







# **Synthesis and Evaluation of Fosmidomycin Analogues as Antimalarial Agents**

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**Thesis submitted to the Faculty of Pharmaceutical Sciences in order to obtain  
the degree of Doctor in the Pharmaceutical Sciences**

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**Academic year 2006-2007**



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## ACKNOWLEDGEMENTS

Dit werk mogen doen was een unieke kans. Ik wil mijn promotor, Prof. Dr. Serge Van Calenbergh oprecht en hartelijk bedanken voor de kans die hij me gaf om dit nobele doctoraat te beginnen en te volmaken. Zijn evaluaties, zijn wetenschappelijke kennis, zijn inzicht in de materie, zijn professionalisme, zijn correcties en zijn beschikbaarheid waren onmisbaar.

I like to thank Dr. Jochen Wiesner for the biological testing and the fast feedback and delivery of information. I thank Dr. Hassan Jomaa and all the members of the European consortium. I would like to express my gratitude to the European Commission for the grants (QLK2-CT-2002-00887) and INTAS (03-51-4077). I sincerely acknowledge Prof. Dr. Schlitzer for the information he provided me to write the introduction of this work.

Ik ben ook dank verschuldigd aan Prof. Dr. Roger Busson (K.U. Leuven) bij wie ik telkens terecht kon voor NMR problemen. Dank je wel voor de uitgebreide studie van sommige NMR stalen en voor de geduldige lessen. Tevens wil ik Prof. Dr. Jef Rozenski (K.U. Leuven) bedanken voor het opnemen van bijna alle massastalen. Ik wil Ir. Jan Goeman (faculteit wetenschappen) bedanken om me te helpen met HPLC en LC-MS opzuiveringen, en ook voor zijn altijd vakkundige uitleg.

Dank je wel aan mijn collega's, waarvan sommige goeie maten geworden zijn. Ineke, Liesbeth, Helga, Veerle, Ulrik, Vincent, Philippe, Stijn, Steven, Matthias, Thomas. Izet, merci voor de steun, de citaten en de filosofie. Jeff (Dr. Carl Lacey), a special word for you. You were my scientific father, but besides chemistry you learned me about life...and life is chemistry. "Never give up, never surrender". You enriched my life. Thank you very much.

Een speciaal woord van dank gaat ook uit naar mijn nonkel (Prof. Dr. Achiël Haemers) voor zijn interesse en raadgevingen.

Ik wil ook Sara Van Poecke en Gert Jan Rotthier bedanken voor de samenwerking gedurende hun thesisjaar.

Collega's binnen de farmacie die vrienden zijn, Michaël, Kevin (Dr. Kevin Huvaere), Bart (Dr. Apr. Bart Synnaeve), Frederik (Dr. Apr. Frederik Roelens), Wim, Thomas, Arne (Dr. Arne Heyerick), Bruno (Dr. Ir. Bruno De Geest), Ruben, Bram, Thomas, Bruno ...merci voor de mooie momenten. Hopelijk volgen er nog vele.

Ik wil vrienden en wijze kennissen bedanken binnen de farmacie en buiten de farmacie. Alain: bedankt voor uw tips en kritiek. Ook zij met wie ik slechts beperkt het zelfde levenspad gedeeld heb wil ik bedanken.

Wat mijn vader zegt is sterk bindend. De wijsheid van mijn vader was een sleutel tot mijn geluk. Mijn moeder was, naast een excellente moeder ook een uitstekend manager. De middenweg die we in ons leven tot nu toe bewandeld hebben was mooi. Ma'tje en Pa, dit moment is goed gelegen om jullie te bedanken voor alles.

Nogmaals dank aan iedereen!

## LIST OF ABBREVIATIONS

ACN	Acetonitrile
ACT	Artemisin-based combination
AFMoC	Adaptation of fields for molecular comparison
Ala	Alanine
ALT	Alanine aminotransferase
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the curve
Bn	Benzyl
<i>t</i> -BOC	<i>tert</i> -Butoxycarbonyl
<i>n</i> -BuLi	<i>n</i> -Butyllithium
<i>t</i> -BuOH	<i>tert</i> -Butanol
cAMP	Cyclic adenosine monophosphate
CDA	Combination Lap-Dap artesunate
CDI	Carbonyl diimidazole
CDP-ME	4-Diphosphocytidyl-2- <i>C</i> -methyl-D-erythritol
CMK	4-Diphosphocytidyl-2- <i>C</i> -methyl-D-erythritol kinase (IspE)
COSY	Correlated spectroscopy
CRP	cAMP receptor protein
Cys	Cysteine
DA	Diode-array
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DEPT	Distortionless Enhancement by Polarization Transfer
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
DMAP	Dimethylaminopyridine
DMAPP	Dimethylallyl pyrophosphate
DMF	Dimethyl formamide

DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DOXP	1-deoxy-D-xylulose 5-phosphate
DXR	1-deoxy-D-xylulose 5-phosphate reductoisomerase (IspC)
DXS	1-deoxy-D-xylulose 5-phosphate synthase
ECG	Electrocardiogram
ee	Enantiomeric excess
EI	Enzyme inhibitor complex
ELSD	Evaporative light scattering detector
ER	Endoplasmatic reticulum
ESI	Electrospray ionisation
Et <sub>3</sub> N	Triethylamine
Et <sub>2</sub> O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
FAS	Fatty acid synthesis
FPIX	Ferriprotoporphyrine IX
<i>fsr</i>	Fosmidomycin resistance
G6PD	6-phosphate dehydrogenase
GFP	Green fluorescent protein
Glu	Glutamate
Gly	Glycine
<i>glp</i>	Glycerol 3-phosphate
His	Histidine
HMBPP	2-hydroxymethyl- <i>cis</i> -but-2-en-4-yl diphosphate
HMGCoA	Hydroxymethylglutaryl coenzyme A
HMPA	Hexamethylphosphorous triamide
HMQC	Heteronuclear multiple quantum coherence
HPLC	High-performance liquid chromatography
IC	Inhibitory concentration
IDI	Isopentenyl diphosphate isomerase
IDS	Isopentenyl diphosphate/dimethylallyldiphosphate synthase (IspH)

Ile	Isoleucine
i.m.	Intramuscular
i.p.	Intraperitoneal
IPP	Isopentenyl pyrophosphate
IUPAC	International union of pure and applied chemistry
i.v.	Intravenous
Lap-Dap	Combination Chlorproguanil-dapsone
LC	Liquid chromatography
LD	Lethal dose
Lys	Lysine
mAu	Milli absorption units
MCPBA	Metachloroperoxybenzoic acid
ME-cPP	2- <i>C</i> -methyl-D-erythritol-2,4-cyclodiphosphate
Mel	Iodomethane
MeOH	Methanol
MEP	2- <i>C</i> -methyl-D-erythritol 4-phosphate
MeSO <sub>2</sub> NH <sub>2</sub>	Methanesulfonamide
Met	Methionine
MIC	Minimum inhibitory concentration
min.	Minutes
MMV	Medicines for malaria venture
MS	Mass spectrum
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
nm	Nanometer
NMR	Nuclear magnetic resonance
NOEDIF	Nuclear overhauser effect difference
NOESY	Nuclear overhauser effect spectroscopy
NPP	New permeability pathways
PIFA	PhI(O–CO–CF <sub>3</sub> ) <sub>2</sub> or Bis(trifluoroacetoxy)iodo benzene
<i>Pf</i> ATP6	<i>Plasmodium falciparum</i> ATP-dependent calcium pump
<i>pfcr</i>	<i>Plasmodium falciparum</i> chloroquine resistance transporter gen
<i>pfmdr</i>	<i>Plasmodium falciparum</i> multi drug resistance gen

p.o.	Per os
ppm	Parts per million
Q/oaTOF	Quadrupole/orthogonal-acceleration time-of-flight
RBC	Red blood cells
rt	Room temperature
SAR	Structure-activity relationship
Ser	Serine
SP	Sulfadoxine pyrimethamine
TBAF	Tetrabutylammonium fluoride
TBDMS	<i>tert</i> -Butyldimethylsilyl
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin layer chromatography
TMSBr	Bromotrimethylsilane
TMSI	Iodotrimethylsilane
Triflate (Tf)	Trifluoromethane sulfonate
Trp	Tryptophan
TsOH	<i>p</i> -Toluenesulfonic acid
UDP	Uridine diphosphate
UTI	Urinary tract infection
UV	Ultraviolet
Val or V	Valine
WBC	White blood cells
WHO	World Health Organisation

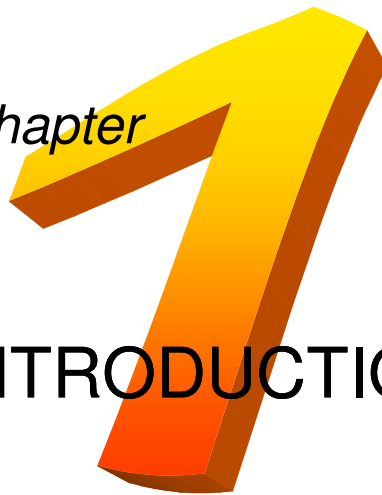






*Chapter*

# INTRODUCTION





# 1. INTRODUCTION

## 1.1. MALARIA

### 1.1.1. ETIOLOGY OF MALARIA

Malaria is a life-threatening disease transmitted by mosquitoes. The disease was supposed to come from fetid marshes, hence the name *mal aria* (bad air). In 1880, scientists discovered the real culprit of malaria is a parasite. Later they discovered that the parasite is transmitted from person to person through the bite of a female mosquito of the genus *Anopheles*, which requires blood to nurture her eggs. *Anopheles gambiae* is the most efficient, so most dreaded species.

Malaria is a protozoan disease caused by unicellular parasites of the genus *Plasmodium*. Four representatives cause the disease in humans:

- *Plasmodium falciparum* (causes malaria tropica)
- *Plasmodium vivax* (malaria tertiana)
- *Plasmodium malariae* (malaria quartana)
- *Plasmodium ovale* (malaria tertiana)

Most attention is directed toward *P. falciparum*. *P. vivax* and *P. falciparum* are the most common, but *P. falciparum* is responsible for the most lethal type of malaria infection. *Plasmodium* belongs to the *Apicomplexa* strain. This phylum is characterized by a cytoplasmic apical complex, that probably facilitates the penetration in the host cell. This unusual plastid-like organelle, also called apicoplast, is acquired through evolution by endosymbiosis of unicellular green algae and is related to the chloroplast of plants.<sup>1</sup> Together with the *Microspora*, the *Apicomplexa* form the *Sporozoa*.

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<sup>1</sup> Jomaa, H., Wiesner, J., Sanderbrand, S., Altincicek, B., Weidemeyer, C., Hintz, M., Turbachova, I., Eberl, M., Zeidler, J., Lichtenthaler, H.K., Soldati, D. and Beck E. *Science* **1999**, 285, 1573-1576.

### 1.1.2. LIFE CYCLE OF *P. FALCIPARUM*

A complex life cycle with different stages (sporozoites, trophozoites,...) and different hosts is often seen in *Sporozoa*. Generally, *Sporozoa* are characterized by the presence of infective stages in their life cycle which are in a spore or transmitted via a vector.

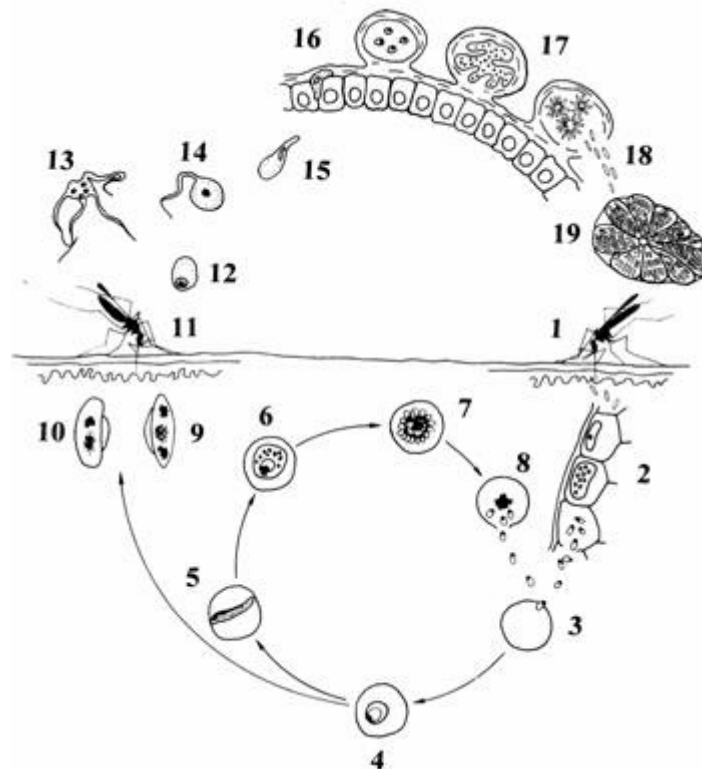


Figure 1.1: The life cycle of *Plasmodium* species: detailed description in the text.

In the case of malaria, the sporozoites enter the human host when an infected *Anopheles* mosquito takes a blood meal (1) (Figure 1.1). First, the sporozoites migrate to the liver where they multiply asexually. Each sporozoite develops into a tissue schizont containing approximately 10000 to 30000 merozoites (2). This process is called the “pre-erythrocytic schizogoni”. These merozoites leave the liver cells and enter the blood stream where they penetrate red blood cells (3). During a 48-hour period asexual reproduction occurs. Successively there are stages of rings (4), trophozoites (5) and blood schizonts (6). In this intra-erythrocytic stage, hemoglobin serves as nourishment for the parasite. In the segmentation stage (7)

(“erythrocytic schizogoni”), every schizont divides into 6-24 merozoites (8). By lysis of red blood cells the new merozoites enter the bloodstream to infect new red blood cells. A small portion of trophozoites undergoes differentiation into female (9) and male (10) gametocytes. The gametocytes are taken up by the mosquito when it bites an infected individual (11). Subsequently, the gametocytes develop in the mid-gut either in macrogametes (♀) (12) or in flagellated microgametes (♂) (13). The male and female gametes fuse and form a zygote (14), which penetrates the gut wall (16) as a motile ookinete (15). The ookinete undergoes a reductive division and some normal divisions towards an oocyst (17) under the external membrane of the mosquito midgut. This oocyst will produce thousands of sporozoites, which are released after rupture of the oocyst (18). The sporozoites migrate to the salivary glands (19) where they are appropriately placed to infect a new individual. In case of *P. vivax* and *P. ovale* some sporozoites turn into hypnozoites, a dormant form which can cause relapses months or years after the initial infection.

### **1.1.3. SYMPTOMS AND DIAGNOSIS OF MALARIA**

Malaria is characterized by extreme exhaustion associated with paroxysms of high fever, sweating, shaking chills, headache, vomiting and anaemia. Malaria symptoms appear about 9 to 14 days after the infectious mosquito bite, although this time space varies with different plasmodium species. The clinical symptoms of malaria are exclusively caused by the erythrocytic parasite stages. With the release of the parasites from infected erythrocytes, cell debris responsible for the characteristic fever spike pattern enters the blood stream. *P. falciparum* produces specific proteins that are embedded in the cell membrane of infected erythrocytes, and as a consequence they stick to the walls of pre-venous capillaries.

If left untreated, the infection can progress rapidly to become life-threatening. Malaria can kill by infecting and destroying red blood cells (anaemia) and by clogging the capillaries that carry blood to the brain (cerebral malaria) or other vital organs. A perilous but treatable complication of malaria is hypoglycaemia due to parasite metabolism in red blood cells.<sup>2</sup> The diagnosis of malaria is set by a blood swab,

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<sup>2</sup> White, N. J., Miller, K. D., Marsh, K., Berry, C. D., Turner, R. C., Williamson, D. H., Brown, J. *Lancet* **1987**, 1, 708-711.

which is subjected to a Giemsa colouring. With this colouring agent the intraerythrocytic stages are visualised (Figure 1.2).<sup>3</sup>

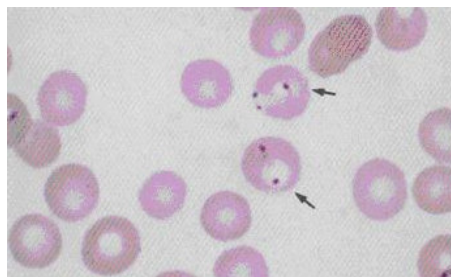


Figure 1.2: Arrows indicate the ring forms (4 in Figure 1.1) of *P. falciparum* in erythrocytes (Giemsa stain, 1000×) (Figure reprinted from “Web Atlas of Medical Parasitology”, <http://atlas.or.kr>).

#### **1.1.4. EPIDEMIOLOGY OF MALARIA**

Today approximately 40% of the world's population, mostly living in the world's poorest countries, are at risk of malaria. The disease was once more widespread, but it was successfully eliminated from many countries with temperate climates during the mid 20th century. Today malaria is found throughout the tropical and sub-tropical regions of the world and causes more than 300 million acute infections. Generally, it is accepted that every year between 700000 and 2.7 million people die of malaria, most of the victims being African children.<sup>4</sup> It is estimated that every 40 seconds a child dies from malaria.<sup>5</sup> However, it is very difficult to obtain exact figures. Malaria is endemic in parts of Asia, Africa, Central and South America, Oceania, and certain Caribbean islands. *P. falciparum* malaria is most common in Africa, south of the Sahara, accounting in large part for the extremely high mortality in this region. There are also worrying indications of the spread of *P. falciparum* malaria into new regions of the world and its reappearance in areas where it had been eliminated. In endemic areas in Africa, every year between 400 and 900 million cases of acute fever by children younger than 5 years, are reported, but how many are caused by malaria is

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<sup>3</sup> Giemsa, G. *Centralblatt für Bakteriologie* **1904**, 32, 307-313.

<sup>4</sup> Snow, R. W., Craig, M., Deichmann, U., Marsh, K. *Bull. World Health Organ.* **1999**, 77, 624-640.

<sup>5</sup> Sachs, J. , Malaney, P. *Nature* **2002**, 415, 680-685.

difficult to estimate. Making a correct diagnosis is indeed a huge problem in these countries. Furthermore, the cases of malaria that are reported are just the tip of the iceberg, or “the ears of the hippopotamus”, if we stay in the African context.<sup>6</sup> The disease has sometimes an asymptomatic course. The extent of the impact of malaria induced anaemia and hypoglycaemia have only recently been appreciated. Also the economical damage, as a result of disease or chronicle brain damage, and children with a low birth weight, as a result of an infected mother, must be considered.

### **1.1.5. MALARIA PROPHYLAXIS**

Apart from drug treatment of infected patients the disease is generally controlled via its vector, the *Anopheles* mosquito. First of all, it is very important to avoid contact with the *Anopheles* mosquito. The mosquitoes are controlled with insecticide spraying and as the mosquito only bites during the night, it is very important to sleep under mosquito nets in risk areas.

Chemoprophylaxis is the second essential strategy in malaria prophylaxis. Drugs are preferably tissue schizonticides, because they kill early development stages of the parasite and prevent the invasion of erythrocytes. However since it is impossible to predict the infection before clinical symptoms begin, this mode of therapy is more theoretical than practical. Today, chemoprophylaxis of malaria relies essentially on four major strategies: chloroquine, proguanil in combination with chloroquine or atovaquone, mefloquine and doxycycline. These drugs are blood schizonticides and will be discussed in paragraph 1.1.7. The risk of an infection remains, since only the parasites in the blood are killed. A combination of measurements is necessary because up to date no antimalarial drug has proven efficient enough to completely prevent malaria.

### **1.1.6. RESISTANCE**

The use of antimalarial drugs to treat fever is an important cause of resistance. Continual exposure of malarial parasite populations to different drugs may have

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<sup>6</sup> Breman, J. G. *Am. J. Trop. Med. Hyg.* **2001**, 64, 1-11.

selected not only for resistance to individual drugs but also for genetic traits that favor initiation of resistance to novel unrelated antimalarials.<sup>7</sup> Of all *Plasmodium* species *P. falciparum* has the highest ability to develop drug resistance.<sup>8</sup> Chloroquine resistant forms of *P. vivax* are also reported, especially in Papua New Guinea and Indonesia. Among the other species no resistance has been documented yet.<sup>9</sup>

The capacity to acquire resistance depends on several factors. First, the mechanism of action is very important. Drugs which target a single enzyme can lose their activity by a single mutation in a gene, the so-called monogenic resistance. Another important factor is the pharmacokinetic profile. A window of opportunity where drug levels fall between sub-therapeutic and sub-selective levels was proposed for certain antimalarial drugs.<sup>10</sup> In this interval, parasites are not killed by the drug, although there is a certain selective pressure for resistance present whereby the resistant strains are selected. Even if all parasites of a first infection are killed, these low plasma concentrations exert a positive selective pressure afterwards. The selective pressure for resistance is related to a long elimination half-life and this behaviour is especially important in areas where the disease is very endemic. The ideal pharmacokinetic profile of an antimalarial drug would comprise a rapid achievement of drug levels above the minimal inhibitory concentration (MIC), maintenance of such drug levels until all parasites from the initial infection have been killed and then the rapid fall of drug levels to sub-selective concentrations.

Also, the efficiency of parasite kill (log-kill rate) is obviously a very important factor since only the surviving part of the parasite population has the opportunity to develop resistance. For this reason combination therapy is useful.

Endemicity is also an important factor. Endemic malaria is correlated with the immunity of the population. The efficacy of chemotherapy will increase in places where the population demonstrates a potent immune response for malaria and, as a result, the opportunity for manifestation and spread of resistant strains will decrease. The challenges in antimalarial drug discovery remain attractive by the rising resistance issue.

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<sup>7</sup> Rathod, P. K., McErlean, T., Lee, P.-C. *Proc. Natl. Acad. Sci. USA* **1997**, 94, 9389-9393.

<sup>8</sup> Winstanley, P. A., Ward, S. A., Snow, R. W. *Microbes Infect.* **2002**, 4, 157-164.

<sup>9</sup> Wongsrichanalai, C., Pickard, A. L., Wernsdorfer, W. H., Meshnik, S. R. *The Lancet Infectious Diseases* **2002**, 2, 209-218.

<sup>10</sup> Watkins, W. M., Mosobo, M. *Trans. Roy. Soc. Trop. Med. Hyg.* **1993**, 87, 75-78.



### 1.1.7. DRUGS AGAINST MALARIA

Malaria was the first disease to be treated with a pure substance (quinine) and the first to be treated with a synthetic drug (methylene blue). Antimalarial agents can be classified in many ways. They can be ranked by the stages of the life cycle which are targeted by the drug. They are divided in: blood schizonticides, tissue schizonticides, hypnozoitocides and gametocytocides. Another possible classification is based on the mechanism of action. In this concise overview established antimalarial drugs as well as agents under developments are discussed. Figure 1.3 shows an erythrocyte infected with a trophozoite. It gives an overview of the targets and the corresponding established and new antimalarial drugs.

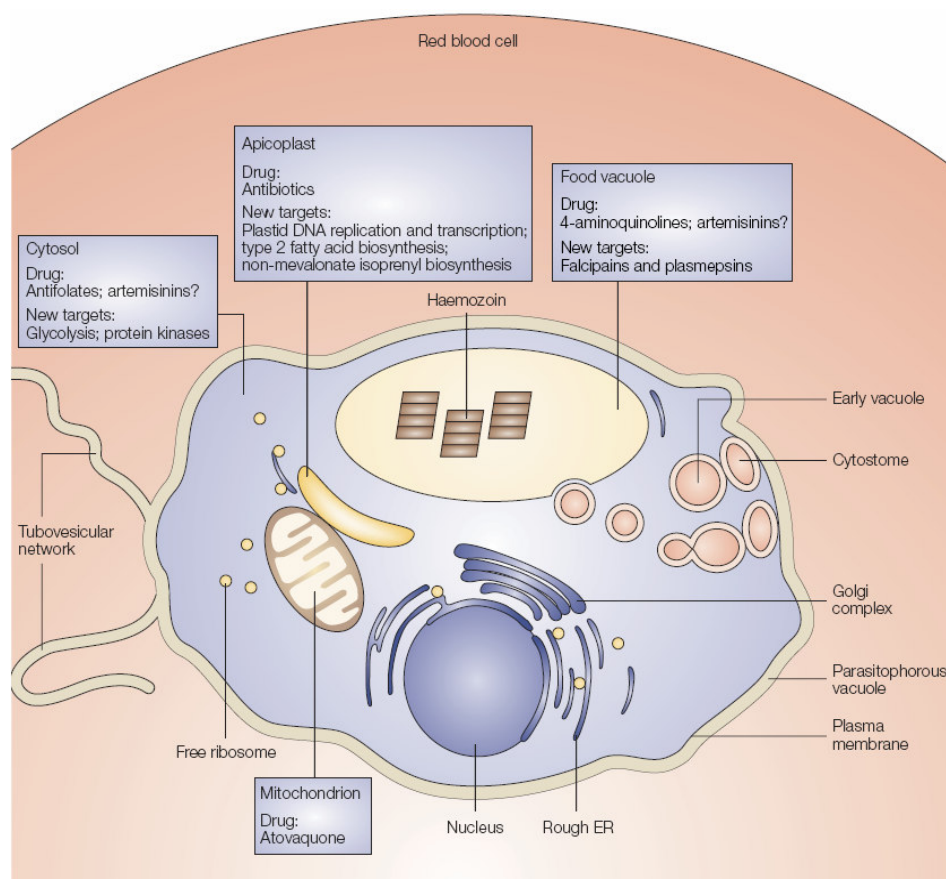


Figure 1.3: Schematic representation of an intra-erythrocytic *P. falciparum* trophozoite, highlighting key parasite intracellular compartments and the site of action of some of the major classes of antimalarial drugs. Efforts are ongoing to develop new drugs against an even wider range of subcellular compartments and parasite targets. (Figure reprinted from *Nature Reviews Drug Discovery* **2004**, 3, 509-520)

### 1.1.7.1. QUININE AND RELATED DERIVATIVES

#### 1.1.7.1.1 4-Aminoquinolines

Chloroquine (**1.1**) (Figure 1.4), a synthetic 4-aminoquinoline, can be considered as one of the most important antimalarials ever. The inhibition of the so-called hem polymerization is generally accepted as mechanism of action. The trophozoite digests small peptides from hemoglobin. As a result, hem is metabolised to ferriprotoporphyrin IX (FPIX) (also called hemozoin),<sup>11</sup> which is toxic for the parasite. Therefore the parasite converts FPIX into hemozoin which accumulates in the food vacuole (Figure 1.3). This can be microscopically seen as the malaria pigment (Red dots in Figure 1.2). The inhibition of hem polymerization, by complexation of 4-aminoquinolines with FPIX, gives rise to harmful hem metabolites which accumulate and destroy the parasite's membrane and thereby compromise cell viability.<sup>12</sup> Hem is also spontaneously oxidized, and some moves to the cytoplasm where it is destroyed by reduced glutathione. The inhibition of this process is considered as an extra mechanism.

The activity of 4-aminoquinolines depends on their basic amino group, which in the acidic food vacuole becomes protonated thereby allowing these derivatives to accumulate. This mechanism is also known as the weak base effect.<sup>13</sup> The main function is however the inhibition of hem polymerization and the oxidative and glutathione-dependent hem degradation. But this remains still a matter of debate.<sup>14,15,16</sup> Chloroquine can also be used as a prophylactic drug.

As a result of addition to table salt, in an attempt toward a global eradication of malaria, the first cases of chloroquine resistance appeared at the end of the 1950s. Nevertheless, it has taken a long time before the first resistance against chloroquine was reported.<sup>17</sup> Chloroquine resistance is linked to multiple factors, including

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<sup>11</sup> Bray, P. G., Ward, S. A., O'Neill, P. M. *Curr. Top. Microbiol. Immunol.* **2005**, 295, 3-38.

<sup>12</sup> Slater, A. F. G. *Pharmacol. Ther.* **1993**, 57, 203-235.

<sup>13</sup> Ginsburg, H., Nissani, E., Krugliak, M. *Biochem. Pharmacol.* **1989**, 38, 2645-2654.

<sup>14</sup> Tilley, L., Loria, P., Foley, M. *The History of Antimalarial Drugs, in: Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery* (Ed.: P. J. Rosenthal), Humana Press Inc., Totowa, 87-121.

<sup>15</sup> Egan, T. J. *Targets* **2003** 3, 115-124.

<sup>16</sup> O'Neill, P. M., Ward, S. A., Berry, N. G., Jeyadevan, J. P., Biagini, G. A., Asadollaly, E., Park, B. K., Bray, P. G. *Curr. Top. Med. Chem.* **2006**, 6, 479-507.

<sup>17</sup> Winstanley, P. A., Ward, S. A., Snow, R. W. *Microbes Infect.* **2002**, 4, 157-164.

changes in transport proteins.<sup>18</sup> The substitution of lysine for threonine in codon 76 of the *pfcr* gene, which codes for a vacuolar membrane transporter protein,<sup>19</sup> is strongly associated with chloroquine resistance.<sup>20</sup> Accumulation of chloroquine in the food vacuole is more pronounced in chloroquine-sensitive strains, then in chloroquine-resistant strains. This observation shows that there is a specific 4-aminoquinoline saturable binding site, which is correlated with the concentration of hematin (FPIX). Saturable chloroquine uptake is due to the binding of chloroquine to hematin rather than active uptake: using Ro 40-4388, a potent plasmepsine I inhibitor of hemoglobin digestion, a reduction in the number of chloroquine binding sites was demonstrated.<sup>21</sup> The link with the *pfcr* gene is not yet elucidated. The consequences of the codon 76 mutation can be neutralized by the use of chloroquine resistance reversers, such as verapamil by restoring the positive charge of lysine. The *pfmdr1* gene codes for *P*-glycoprotein homologue 1, another membrane transporter. Polymorphism in the *pfmdr1* gene, for instance an aspartic acid to tyrosine point mutation in codon 86, has been associated with chloroquine resistance in some countries, but not in others, so the evidence has not been convincing. Mutations of this gene would also be responsible for mefloquine (*vide infra*) and quinine resistance. Moreover, chloroquine resistant parasites are less sensitive to quinine (*vide infra*).<sup>22,23</sup>

Chloroquine resistance is also linked to a higher content of reduced glutathione in the parasite;<sup>24</sup> therefore, new combination drugs have been designed (*vide infra*). In spite of this, chloroquine still represents the most frequently used antimalarial drug owing to its high tolerance and low production costs. However, as a result of the worldwide spread of severe resistance to chloroquine more than ten African countries have switched their first-line treatment to sulfadoxine-pyrimethamine which useful lifespan is also more or less terminated (see 1.1.7.3.). As a prophylactic agent, chloroquine can only be administered as monotherapy in areas where no resistance has been reported.

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<sup>18</sup> Mutabingwa, T., Nzila, A., Mberu, E., Nduati, E., Winstanley, P., Hills, E., Watkins, W. *Lancet* **2001**, 358, 1218-1223.

<sup>19</sup> Welles, T. E., Walker-Jonah, A., Pantom, L. J. *Proc. Natl. Acad. Sci. USA* **1991**, 88, 3382-3386.

<sup>20</sup> Wongsrichanalai, C., Pickard, A. L., Wernsdorfer, W. H., Meshnik, S. R. *The Lancet Infectious Diseases* **2002**, 2, 209-218.

<sup>21</sup> Bray, P. G., Mungthin, M., Ridley, R. G., Ward, S. A. *Mol. Pharmacol.* **1998**, 54, 170-179.

<sup>22</sup> Zalis, M. G., Pang, L., Silveira, M. S., Milhous, W. K., Wirth, D. F. *Am. J. Trop. Med. Hyg.* **1998**, 58, 630-637.

<sup>23</sup> Le Bras, J., Durand, R. *Fundam. Clin. Pharmacol.* **2003**, 17, 147-153.

<sup>24</sup> Atamna, H., Ginsburg, H. *J. Biol. Chem.* **1995**, 270, 24876-24883.

Due to some reported cases of agranulocytosis in the mid 1980s, the use of the related amodiaquine (**1.2**) has been limited. There is however a renewed interest in using this drug, because of its efficacy, especially against low-level chloroquine-resistant *P. falciparum*.<sup>25</sup> Amodiaquine is among the antimalarial drugs currently recommended by the WHO.<sup>26</sup>

Pyronaridine (**1.3**) is structurally related to chloroquine and amodiaquine and is a very active 4-aminoquinoline derivative. However, it is registered only on the Chinese market, due to failure to meet international regulatory standards.<sup>27</sup> Most likely, it also acts as an inhibitor of hem polymerization.

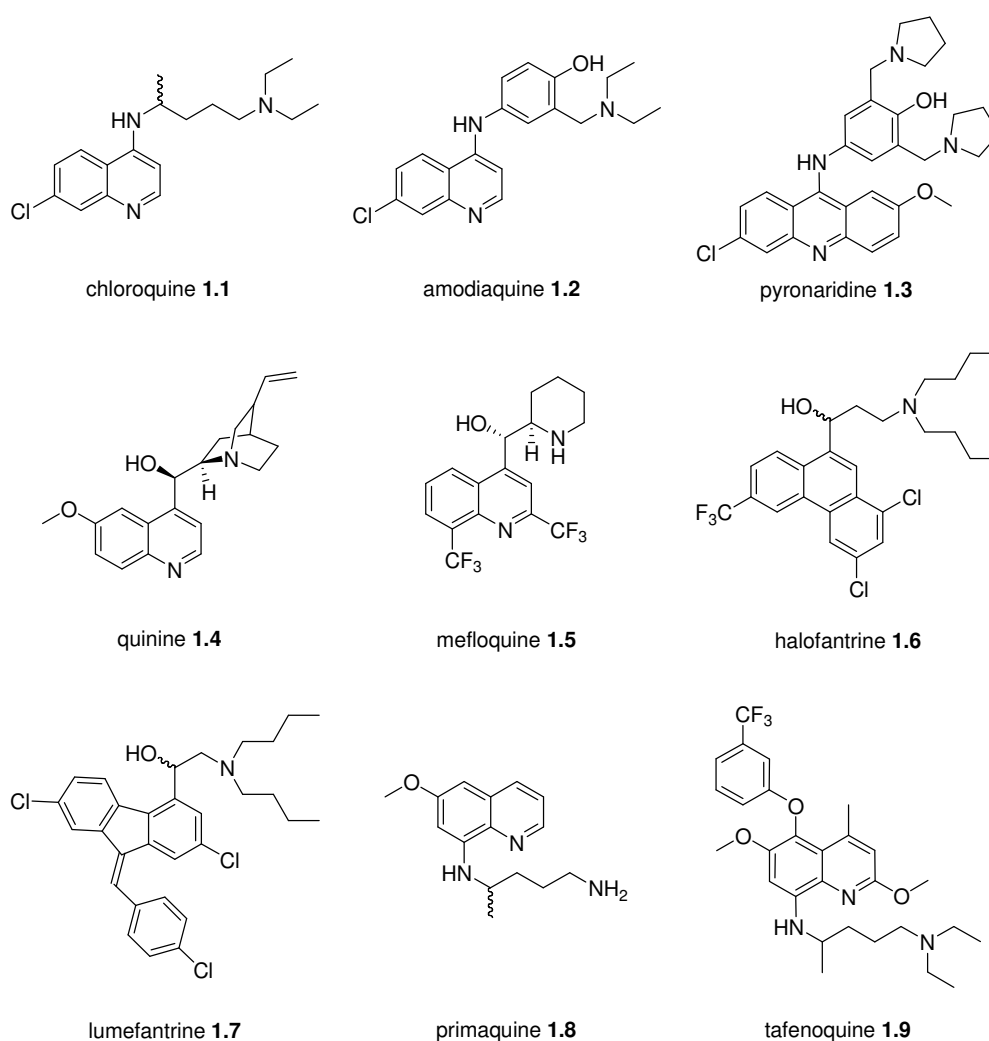


Figure 1.4: Structures of quinine and derivatives.

<sup>25</sup> Ridley, R. G. *Nature* **2002**, 415, 686-693.

<sup>26</sup> <http://www.who.int/malaria/docs/TreatmentGuidelines2006.pdf>

<sup>27</sup> Olliaro, P. L., Milhous, W. K. in *Antimalarial Chemotherapy* (Ed.: Rosenthal, P. J.), Humana press, Totowa, NJ, **2001**, 219-232.

#### 1.1.7.1.2 Quinine and arylaminoalcohols

Quinine (**1.4**) (Figure 1.4), the principal alkaloid of the Chinchona bark, has already been used in the treatment of malaria and fever for 350 years. Since the nineteen thirties, synthetic drugs derived from quinine have been developed, but quinine still plays an important role in the treatment of multiresistant malaria.

The mechanism of action of quinine and other arylaminoalcohols is probably different from the mechanism of 4-aminoquinolines. Recently, it has been proposed that arylaminoalcohols prevent the fusion of haemoglobin shuttling vesicles to the food vacuole.<sup>28</sup> Quinine is also toxic against schizonts and against gametocytes of *P. malariae* and *P. vivax*. In most areas quinine is still the only drug available for intravenous treatment of severe malaria. Its diastereomer quinidine is 2-3 fold more active, but cannot be used as antimalarial drug because of cardiac toxicity.<sup>29</sup>

Mefloquine (**1.5**), an arylaminoalcohol, is the reference drug for treating chloroquine-resistant malaria. Unfortunately, it suffers from a plethora of side effects (headache, palpitations, vertigo, anxiety, hallucinations, insomnia) and cannot be administered in the first trimester of pregnancy. Mefloquine can be used as a prophylactic drug in chloroquine-resistant areas, but is expensive and the neuropsychiatric side effects are very annoying.

Halofantrine (**1.6**) is highly effective. It is toxic for schizonts and gametocytes, but its use is strongly restricted by its potential to induce heart arrhythmias.<sup>30</sup> The *N*-desbutyl analogue appeared less cardiotoxic than halofantrine.<sup>31</sup>

Lumefantrine (**1.7**) is structurally similar to halofantrine, but induces fewer cardiotoxic side effects.<sup>32</sup> Although its antimalarial activity is weaker than halofantrine, it is administered in a combination with arthemether (*vide infra*). *In vitro* the *N*-desbutyl derivative is fourfold more active than the parent drug.<sup>33,34</sup>

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<sup>28</sup> Fitch, C. D. *Life. Sci.*, **2004**, 74, 1957-1972.

<sup>29</sup> Karle, J. M., Karle, I. L., Gerena, L., Milhous, W. K. *Antimicrob. Agents Chemother.* **1992**, 36, 1538-1544.

<sup>30</sup> Newton, P., White, N. *Annu. Rev. Med.* **1999**, 50, 179-192.

<sup>31</sup> Wesche, D. L., Schuster, B. G., Wang, B. X., Woosley, R. L. *Clin. Pharmacol. Ther.* **2000**, 67, 521-529.

<sup>32</sup> van Agtmael, M., Bouchaud, O., Malvy, D., Delmont, J., Danis, M., Barette, S., Gras, C., Bernard, J., Touze, J.E., Gathmann, I., Mull, R. *Int. J. Antimicrob. Agents* **1999**, 12, 159-169.

<sup>33</sup> Basco, L. K., Peytavin, G., Gimenez, F., Genissel, B., Farinotti, R., Le Bras, J. *Trop. Med. Parasitol.* **1994**, 45, 45-46.

<sup>34</sup> Noedl, H., Allmendinger, T., Prajakwong, S., Wernsdorfer, G., Wernsdorfer, W. H. *Antimicrob. Agents Chemother.* **2001**, 45, 2106-2109.

A main factor in the resistance against mefloquine, halofantrine and lumefantrine is the increase in copy numbers of the *pfmdr1* gene coding for a membrane transporter (*PfMDR1*) which transports arylaminoalcohols into the food vacuole.<sup>35,36,37,38,39</sup>

#### 1.1.7.1.3 8-Aminoquinolines

Primaquine (**1.8**, Figure 1.4) is an 8-aminoquinoline that has been used since the 1940s for the eradication of liver stages.<sup>40</sup> Against blood stages, however, primaquine is inactive at pharmacologically achievable concentrations. Toxicological concerns have led to restrictions in the use of primaquine. The major disadvantage of the 8-aminoquinolines is the induction of hemolytic anaemia in patients with glucose 6-phosphate dehydrogenase (G6PD) deficiency as a potentially life-threatening adverse effect. The incidence of this genetic anomaly is particularly high in areas where malaria is endemic.

Tafenoquine (**1.9**) is a derivative of primaquine. This 8-aminoquinoline is less toxic than primaquine, has a longer plasma half-life and is active against blood and liver stages. The mode of action against blood stages is probably due to the inhibition of hem polymerization. The activity against liver stages is probably related to toxic metabolites which affect mitochondrial processes.<sup>41</sup> Nevertheless, the real mechanism of action of 8-aminoquinolines stays unclear.

#### 1.1.7.1.4 New chloroquine analogues and hem polymerization inhibitors

The extensive use of chloroquine led to the existence of resistant strains. However, the research for new chloroquine derivatives is still actual, since the emergence of chloroquine-resistant strains is rare.<sup>42</sup> This is probably because the

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<sup>35</sup> White, N. J. *J. Clin. Invest.* **2004**, *113*, 1084-1092.

<sup>36</sup> Duraisingh, M. T., Cowman, A. F. *Acta Trop.* **2005**, *94*, 181-190.

<sup>37</sup> Uhlemann, A.-C., Ramharter, M., Lell, B., Kremsner, P. G., Krishna, S. *J. Infect. Dis.* **2005**, *192*, 1830-1835.

<sup>38</sup> Uhlemann, A.-C., Krishna, S. *CTMI* **2005**, *295*, 39-53.

<sup>39</sup> Woodrow, C. J., Krishna, S. *Cell. Mol. Life. Sci.* **2006**, *63*, 1586-1596.

<sup>40</sup> Shanks, G. D., Kain, K. C., Keystone, J. S. *Clin. Infect. Dis.* **2001**, *33*, 381-385.

<sup>41</sup> Brueckner, R. P., Ohrt, C., Braid, J. K., Milhous, W. in *Antimalarial Chemotherapy* (Ed.: Rosenthal, P. J.), Humana press, Totowa, NJ, **2001**, 123-125.

<sup>42</sup> Wellems, T. E., Plowe, C. V. *J. Infect. Dis.* **2001**, *184*, 770-776.

hem polymerization seems not to depend on any enzymes and as a consequence a simple mutation cannot cause resistance.<sup>43</sup>

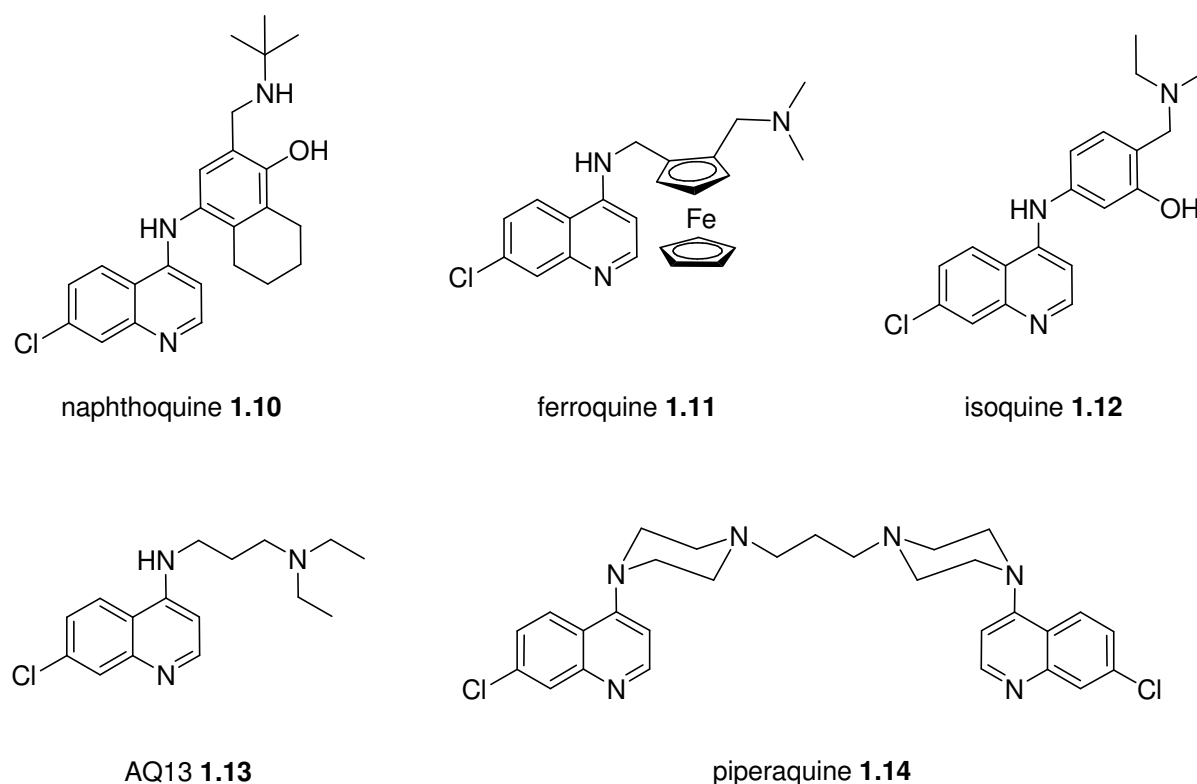


Figure 1.5: New chloroquine derivatives.

Incorporation of an aromatic group in the side chain of chloroquine, as present in amodiaquine (**1.3**) and pyronaridine (**1.4**), resulted in analogues which overcome chloroquine resistance. Naphthoquine (**1.10**), ferroquine (**1.11**) and isoquine (**1.12**) are examples of newer chloroquine analogues (Figure 1.5). Shortening of the diamino alkyl side chain (e.g., AQ13, **1.13**) and dimerisation (e.g., piperazine, **1.14**) are two other reported options to overcome chloroquine resistance.<sup>11,16,44,45,46</sup>

<sup>43</sup> Sanchez, C. P., Lanzer, M. *Curr. Opin. Infect. Dis.* **2000**, *13*, 653-658.

<sup>44</sup> Stocks, P. A., Raynes, K. J., Ward, S. A. The History of Antimalarial Drugs, in: *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery* (Ed.: P. J. Rosenthal), Humana Press Inc., Totowa, 235-253.

<sup>45</sup> O'Neill, P. M., Bray, P. G., Hawley, S. R., Ward, S. A., Park, B. K. *Pharmacol. Ther.*, **1998**, *77*, 29-58.

<sup>46</sup> Foley, M., Tilley, L. *Pharmacol. Ther.*, **1998**, *78*, 55-87.

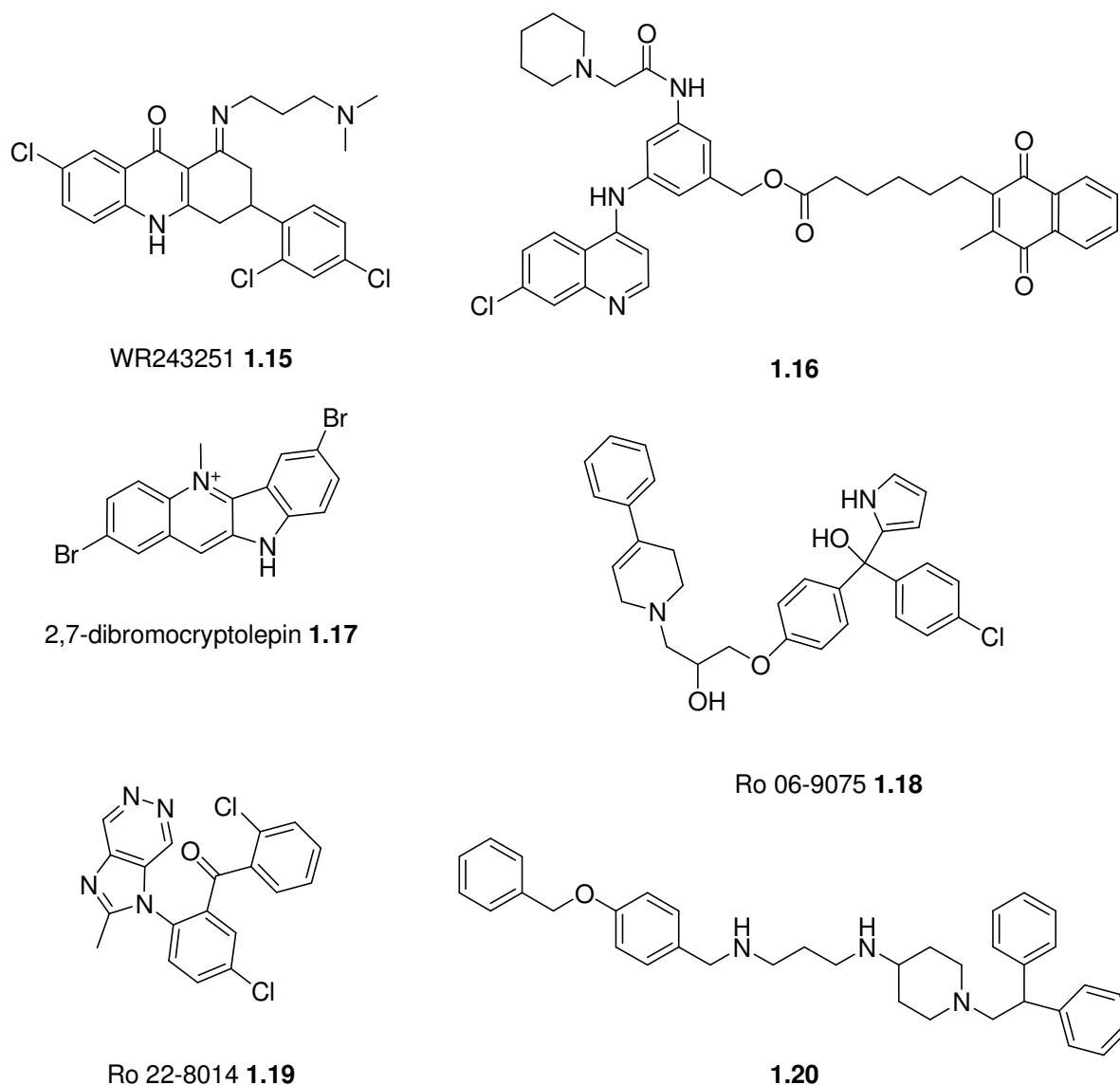


Figure 1.6: Structures of new hem polymerization inhibitors.

WR 243251 (**1.15**, Figure 1.6) is a derivative of mepacrine. Studies demonstrated that **1.15** was active against a chloroquine-resistant *P. falciparum* strain in monkeys. It also inhibits the development of *P. vivax* sporozoites in mosquitoes.<sup>47,48</sup> The mechanism of action is probably dual: the inhibition of the hem polymerization and the inhibition of the parasitic cellular respiration.<sup>49,50</sup>

<sup>47</sup> Berman, J., Brown, L., Miller, R., Andersen, S. L., McGreevy, P., Schuster, B. G., Ellis, W., Ager, A., Rossan, R. *Antimicrob. Agents Chemother.* **1994**, 38, 1753-1756.

<sup>48</sup> Coleman, R. E., Polsa, N., Eikarat, N., Kollars Jr., T. M., Sattabongkot, J. *Am. J. Trop. Med. Hyg.* **2001**, 65, 214-218.

<sup>49</sup> Dorn, A., Scovill, J. P., Ellis, W. Y., Matile, H., Ridley, R. G., Vennerstrom, J. L. *Am. J. Trop. Med. Hyg.* **2001**, 65, 19-20.

<sup>50</sup> Suswan, E., Kyle, D., Lang-Unnasch, N. *Exp. Parasitol.* **2001**, 98, 180-187.



Compound **1.16** is an example of a conjugate consisting of two active molecules linked by an ester linkage, which can be cleaved by esterases to release the active species, i.e. a *P. falciparum* glutathione reductase inhibitor and a 4-aminoquinoline.<sup>51</sup> Several atypical compounds were developed, amongst which many synthetic derivatives of cryptolepine. 2,7-Dibromocryptolepine (**1.17**) emerged as the most active compound. Also here, this activity mostly relies on the inhibition of the hem polymerization.<sup>52</sup>

Several atypical compounds, such as **1.18** and **1.19**, were discovered as hem polymerization inhibitors using extensive screening by *in vitro* microassays.<sup>53</sup> Another class of novel hem polymerization inhibitors, such as compound **1.20**, was originally designed as protease inhibitors.<sup>54,55</sup> Their potential is questioned because of cross-resistance with chloroquine.

### 1.1.7.2. ARTEMISININ DERIVATIVES

#### 1.1.7.2.1 Artemisinin and first generation semi-synthetic artemisinins

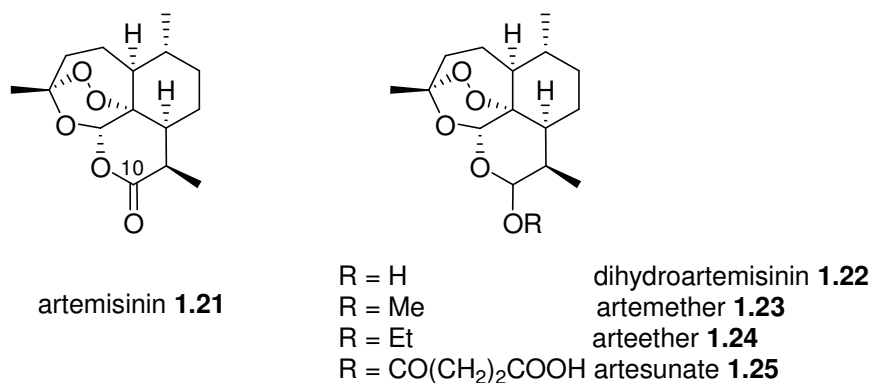


Figure 1.7: Artemisinin and derivatives.

<sup>51</sup> Davioud-Charvet, E., Delarue, S., Biot, C., Schwobel, B., Boehme, C. C., Mussigbrodt, A., Maes, L., Sergheraert, C., Grellier, P., Schirmer, R. H., Becker, K. *J. Med. Chem.* **2001**, *44*, 4268-4276.

<sup>52</sup> Wright, C. W., Addae-Kyereme, J., Breen, A. G., Brown, J. E., Cox, M. F., Croft, S. L., Gokcek, Y., Kendrick, H., Phillips, R. M., Pollet, P. L. *J. Med. Chem.* **2001**, *44*, 3187-3194.

<sup>53</sup> Kurosawa, Y., Dorn, A., Kitsuyi-Shirane, M., Shimada, H., Satoh, T., Matile, H., Hofheinz, W., Masciadri, R., Kansy, M., Ridley, R. G. *Antimicrob. Agents Chemother.* **2000**, *44*, 2638-2644.

<sup>54</sup> Brinner, K. M., Kim, J. M., Habashita, H., Gluzman, I. Y., Goldberg, D. E., Ellman, J. A. *Bioorg. Med. Chem.* **2002**, *10*, 3649-3661.

<sup>55</sup> Choi, C. Y. H., Schneider, E. L., Kim, J. M., Gluzman, I. Y., Goldberg, D. E., Ellman, J. A., Marletta, M. A. *Chem. Biol.* **2002**, *9*, 881-889.

The plant *Artemisia annua*, with the sesquiterpene lactone artemisinin (**1.21**) as an active ingredient has been used for at least 2000 years in traditional Chinese medicine and since 1596 for the treatment of fever. Semi-synthetic artemisinin derivatives artemether (**1.23**), arteether (**1.24**) and artesunate (**1.25**) have been increasingly used for about 20 years, and all are prodrugs which are metabolized to the main active compound dihydroartemisinin (**1.22**) (Figure 1.7). Dihydroartemisinin is a mixture of  $\alpha$ - and  $\beta$ -epimers whose ratio depends on both the reaction conditions and the choice of solvents.<sup>56</sup> Artesunate is the hemisuccinate ester of dihydroartemisinin and exists only as  $\alpha$ -epimer. It is prepared by treatment of dihydroartemisinin with succinic acid in the presence of DMAP.<sup>57</sup> Artemether and arteether are obtained by methylation or ethylation of dihydroartemisinin with the formation of the two epimers in a ratio of  $\alpha$ : $\beta$  22:78.<sup>56</sup>

The artemisinin derivatives act faster than any other antimalarial drug and are the most active antimalarial drugs known today. As a result of the short plasma half-life, the artemisinins are increasingly combined with other antimalarial drugs with longer plasma half-lives, such as mefloquine. These combinations decrease the risk to induce resistance and since 2001 the WHO condemns the use of artemisinins in monotherapy. Other artemisin-based combination treatments (ACT's) are artesunate with amodiaquine, artemether with lumefantrine and dihydroartemisinin in combination with piperaquine.

The artemisinins are active against gametocytes. The biological activity depends on the cleavage of the crucial peroxide bond after contact with  $\text{Fe}^{2+}$ -hem inside the food vacuole, thus generating free radicals that can alkylate the hem molecule.<sup>58</sup> By this mechanism, the detoxification of free hem may be inhibited.<sup>59</sup> Alkylation of essential parasite proteins may contribute to the antimalarial activity.<sup>60</sup> The alkylation properties of peroxides could imply a limitation in the antimalarial use. Not surprisingly, these compounds also attract attention as anticancer drugs.

Another recent theory is the activation of artemisinin by  $\text{Fe}^{2+}$  in the cytoplasm. This process would take place close to an ATP-dependent calcium pump (*Pf*ATP6)

<sup>56</sup> Haynes, R. K., Chan, H.-W., Cheung, M.-K., Lam, W.-L., Soo, M.-K., Tsang, H.-W., Voerste, A., Williams, I. D. *Eur. J. Org. Chem.* **2002**, 1, 113-132.

<sup>57</sup> Utzinger, J., Shuhua, X., Keiser, J., Minggan, C., Jiang, Z., Tanner, M. *Curr. Med. Chem.* **2001**, 8, 1841-1859.

<sup>58</sup> Olliaro, P. L., Haynes, R. K., Meunier, B., Yuthavong, Y. *Trends Parasitol.* **2001**, 17, 122-126.

<sup>59</sup> Cazelles, J., Robert, A., Meunier, B. *J. Org. Chem.* **2002**, 67, 609-619.

<sup>60</sup> Bisutthibhan, J., Pan, X. Q., Hossler, P. A., Walker, D. J., Yowell, C. A., Carlton, J., Dame, J. B., Meshnick, S. R. *J. Biol. Chem.* **1998**, 273, 16192-16198.

located at the endoplasmatic reticulum. This pump would be inhibited by irreversible binding of the activated artemisinin, restraining parasite growth.<sup>61</sup> How the inhibition of *Pf*ATP6 leads to the rapid killing of the parasite is unknown. There have been no clinical reports on artemisinin resistance. Similarly, as discussed for the 4-aminoquinolines, this might be explained by the fact that no enzyme is involved in the major mode of action. However, it must be mentioned that it has been possible to create a *Pf*ATP6 resistant against artemisinins by the exchange of just one amino acid (Leu263Glu).<sup>62</sup>

A combination of lumefantrine (**1.7**) and artemether (**1.23**) has recently been approved as co-artemether or Riamet®.<sup>63</sup> Both compounds inhibit hem detoxification in the food vacuole resulting in a synergistic effect.<sup>64</sup>

Since a synergistic effect of pyronaridine with artemisinin has been demonstrated *in vitro*,<sup>65</sup> research of a combination therapy involving pyronaridine-artesunate is in progress.<sup>66</sup>

#### 1.1.7.2.2 Second generation semisynthetic artemisinines and synthetic endoperoxides

Although artemisinin itself and several semi-synthetic derivatives are in use today, concerns exist about their high cost of production, low oral activity and the duration of administration these agents require. A major drawback of the currently available semisynthetic artemisinin derivatives is that they are prone to hydrolysis, resulting in a short biological half-life and the generation of the potentially neurotoxic dihydroartemisinin (**1.22**). The search for new synthetic peroxide drug candidates is in progress.<sup>67</sup>

<sup>61</sup> Eckstein-Ludwig, U., Webb, R. J., Van Goethem, I. D. A., East, J. M., Lee, A. G., Klimura, M., O'Neill, P. M., Bray, P. G., Ward, S. A., Krishna, S. *Nature* **2003**, 424, 957-961.

<sup>62</sup> Uhlemann, A.-C., Cameron, A., Eckstein-Ludwig, U., Fischbarg, J., Iserovich, P., Zuniga, F. A., East, M., Lee, A., Brady, L., Haynes, R. K., Krishna, S. *Nature Struct. Mol. Biol.* **2005**, 12, 628-629.

<sup>63</sup> Omari, A. A., Preston, C., Garner, P. *Cochrane Database Syst. Rev.* **2002**, 3, CD003125.

<sup>64</sup> Hassan Alin, M., Bjorkman, A., Wernsdorfer, W. H. *Am. J. Trop. Med. Hyg.* **1999**, 61, 439-445.

<sup>65</sup> Gupta, S., Thapar, M. M., Mariga, S. T., Wernsdorfer, W. H., Bjorkman, A. *Exp. Parasitol.* **2002**, 100, 28-35.

<sup>66</sup> MMV-Medicines for Malaria Venture, Annual report **2000**.

<sup>67</sup> Vennerstrom, J. L., Arbe-Barnes, S., Brun, R., Charman, S. A., Chiu, F. C. K., Chollet, J., Dong, Y., Dorn, A., Hunziker, D., Matile, H., McIntosh, K., Padmanilayam, M., Santo Tomas, J., Scheurer, C., Scorneaux, B., Tang, Y., Urwyler, H., Wittlin S., Charman W. N. *Nature* **2004**, 430, 900-904.

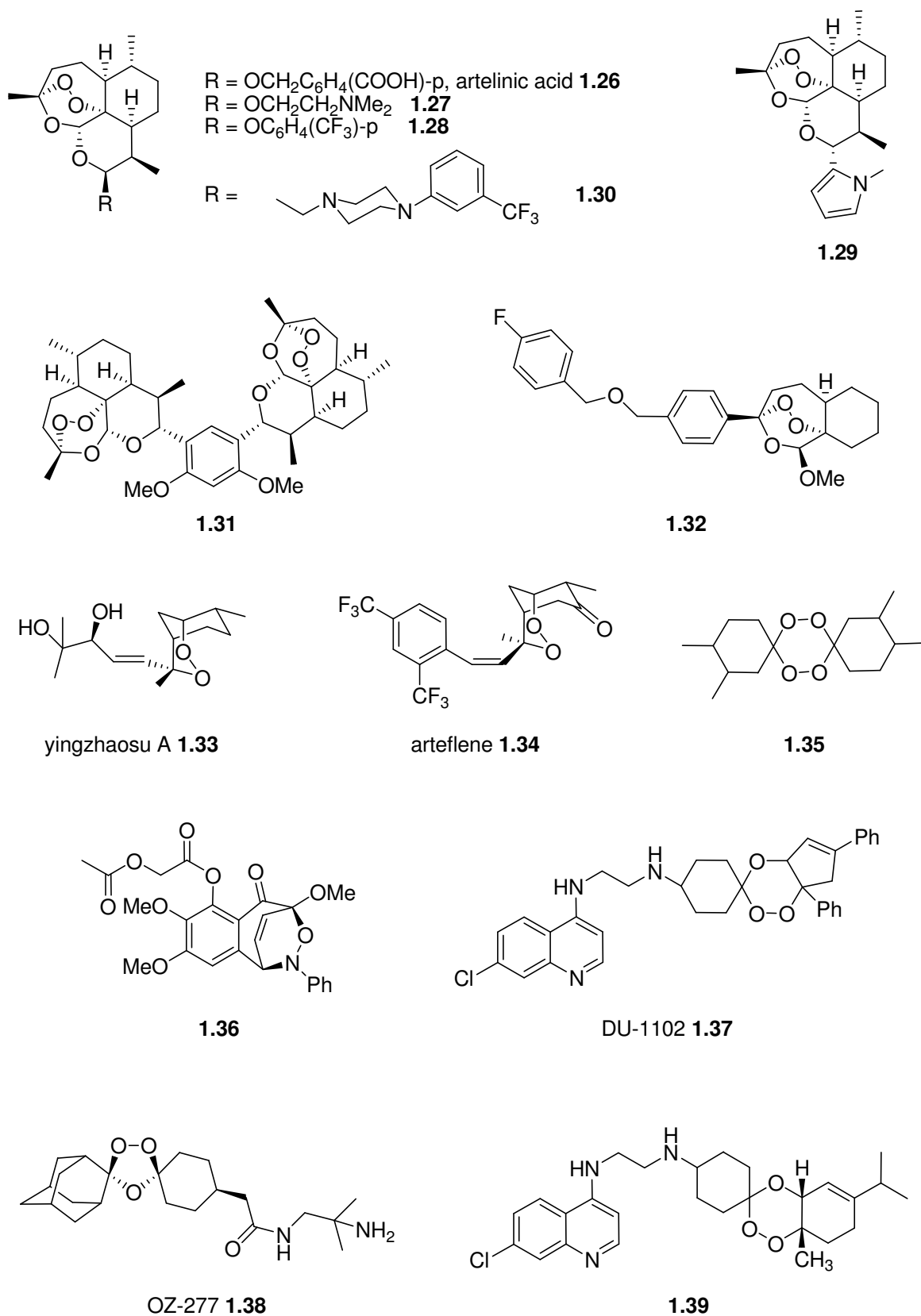


Figure 1.8: Structures of new artemisinin derivatives.

To remedy the major drawbacks of artemisinins, considerable effort has gone into the design of more stable derivatives with improved bioavailability. Most variations address position 10 where the exocyclic oxygen has been substituted for several substituents. Alkyl, aryl and heteroaryl residues have been placed at this position (Figure 1.8). Derivatives **1.26**<sup>68</sup> and **1.27**<sup>69</sup> are highly water soluble and compound **1.26** proved more stable than artesunate. Both were developed for intravenous administration. The *p*-trifluoromethylphenoxy derivative **1.28** proved to be superior to artesunate after oral application in a malaria-infected mouse model.<sup>70</sup> Additional robust and orally active derivatives such as **1.29** were obtained by non-acetal linkage of the side chain to the artemisinin core.<sup>71</sup> It was further rationalized that a piperazine ring in the side chain, such as in compound **1.30**, may lead to enrichment of the drug inside the food vacuole mediated by the weak base effect.<sup>72</sup> Furthermore, modifications have also been applied to other positions. Several reviews cover this issue in depth.<sup>73</sup> Also, artemisinin-derived trioxane dimers, such as compound **1.31**, containing various linkers were synthesized.<sup>74,75</sup> Simplification of the artemisinin core led to synthetic 3-aryl trioxanes, such as **1.32**.<sup>76</sup> Upon oral application in a murine model, **1.32** was twice as active as artemisinin.<sup>77</sup>

<sup>68</sup> Li, Q. G., Peggs, J. O., Lin, A. J., Masonic, K. J., Trotman, K. M., Brewer, T. G. *Trans. R. Soc. Trop. Med. Hyg.* **1998**, *92*, 332-340.

<sup>69</sup> Li, Y., Zhu, Y. M., Jiang, H. J., Pan, J. P., Wu, G. S., Wu, J. M., Shi, Y. L., Yang, J. D., Wu, B. A. *J. Med. Chem.* **2000**, *43*, 1635-1640.

<sup>70</sup> O'Neill, P. M., Miller, A., Bishop, L. P., Hindley, S., Maggs, J. L., Ward, S. A., Roberts, S. M., Scheinmann, F., Stachulski, A. V., Posner, G. H., Park, B. K. *J. Med. Chem.* **2001**, *44*, 58-68.

<sup>71</sup> Posner, G. H., Parker, M. H., Northrop, J., Elias, J. S., Ploypradith, P., Xie, S., Shapiro, T. A. *J. Med. Chem.* **1999**, *42*, 300-304.

<sup>72</sup> Hindley, S., Ward, S. A., Storr, R. C., Searle, N. L., Bray, P. G., Park, B. K., Davies, J., O'Neill, P. M. *J. Med. Chem.* **2002**, *45*, 1052-1063.

<sup>73</sup> (a) O'Neill, P. M. *Expert Opin. Invest. Drugs* **2005**, *14*, 1117-1128. (b) Posner, G. H., Krasavin, M., McCutchen, M., Ploypradith, P., Maxwell, J. P., Elias, J. S., Parker, M. H. *The History of Antimalarial Drugs, in: Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery* (Ed.: P. J. Rosenthal), Humana Press Inc., Totowa, 255-263. (c) Ploypradith, P. *Acta Trop.* **2004**, *89*, 329-342. (d) Borstnik, K., Paik, I., Shapiro, T. A., Posner, G. H. *Int. J. Parasitol.* **2002**, *32*, 1661-1667. (e) Vroman, J. A., Alvim-Gaston, M., Avery, M. A. *Curr. Pharm. Des.* **1999**, *5*, 101-138. (f) O'Neill, P. M., Posner, G. H. *J. Med. Chem.* **2004**, *47*, 2945-2964.

<sup>74</sup> Posner, G. H., Ploypradith, P., Parker, M. H., O'Dowd, H., Woo, S. H., Northrop, J., Krasavin, M., Dolan, P., Kensler, T. W., Xie, S., Shapiro, T. A. *J. Med. Chem.* **1999**, *42*, 4275-4280.

<sup>75</sup> Posner, G. H., Northrop, J., Paik, I. H., Borstnik, K., Dolan, P., Kensler, T. W., Xie, S., Shapiro, T. A. *Bioorg. Med. Chem.* **2002**, *10*, 227-232.

<sup>76</sup> Posner, G. H., Jeon, H. B., Parker, M. H., Krasavin, M., Paik, I. H., Shapiro, T. A. *J. Med. Chem.* **2001**, *44*, 3054-3058.

<sup>77</sup> Posner, G. H., Cumming, J. N., Woo, S. H., Ploypradith, P., Xie, S., Shapiro, T. A. *J. Med. Chem.* **1998**, *41*, 940-951.

Based on the hypothesis that the mechanism of the artemisinins depends on the generation of free radicals by cleavage of the peroxide function, it can be assumed that other endoperoxides not related to the artemisinin structure may also exhibit antimalarial activity. Consequently, yingzhaosu A (**1.33**), which similarly to artemisinin was isolated from a traditional Chinese medicinal plant (*Artabotrys uncinatus*), and the synthetic derivative arteflene (**1.34**) were active against malaria.<sup>78</sup> After successful preclinical trials, arteflene (**1.34**) has already been tested on patients with uncomplicated malaria in Africa.<sup>79</sup> However, its moderate clinical efficacy and difficult synthesis prevented its further development.<sup>80</sup> The development of antimalarial endoperoxides has recently been comprehensively reviewed.<sup>81</sup>

More readily accessible are 1,2,4,5-tetraoxanes such as compound **1.35**, which differ significantly from artemisinin.<sup>82</sup> After oral application to mice infected with *P. berghei*, compound **1.35** was slightly more active than artemisinin, but less active than arteether. Furthermore, different oxazines were synthesized as potential antimalarial drugs. The standard dissociation energy of the N–O bond is similar to that of the O–O bond, the N–O bond is thus prone to undergo homolytic cleavage, resulting in the generation of radicals. As expected, some of these compounds exhibited significant *in vitro* activity against *P. falciparum*. By choosing a suitable substitution pattern, a relatively low cytotoxicity was achieved.<sup>83</sup> The growth of *P. falciparum* was inhibited by compound **1.36** (IC<sub>50</sub> = 3.2 µM).

DU-1102 (**1.37**) is the most active example of a class of hybrids between new artemisinin-like peroxides and a 4-aminoquinoline.<sup>84</sup> These so-called trioxaquinines

<sup>78</sup> Szpilman, A. M., Korshin, E. E., Hoos, R., Posner, G. H., Bachi, M. D. *J. Org. Chem.* **2001**, *66*, 6531-6540.

<sup>79</sup> (a) Weidekamm, E., Dumont, E., Jaquet, C. *Trop. Med. Parasitol.* **1994**, *45*, 278-283. (b) Salako, L. A., Guiguemde, R., Mittelholzer, M. L., Haller, L., Sorenson, F., Sturchler, D. *Trop. Med. Parasitol.* **1994**, *45*, 284-287. (c) Somo-Moyou, R., Mittelholzer, M. L., Sorenson, F., Haller, L., Sturchler, D. *Trop. Med. Parasitol.* **1994**, *45*, 288-291. (d) Radloff, P. D., Philipps, J., Nkeyi, M., Sturchler, D., Mittelholzer, M. L., Kremsner, P. G. *Am. J. Trop. Med. Hyg.* **1996**, *55*, 259-262.

<sup>80</sup> Posner, G. H., Kravasin, M., McCutchen, M., Ploypradith, P., Maxwell, J. P., Elias, J. S., Parker, M. H. in *Antimalarial Chemotherapy* (Ed.: P. J. Rosenthal), Humana Press, Totowa, NJ, **2001**, pp. 255-263.

<sup>81</sup> See reference 73 (a), (b), (c), (d), (f) and Tang, Y., Dong, Y., Vennerstrom, J. L. *Med. Res. Rev.* **2004**, *24*, 425-448.

<sup>82</sup> Vennerstrom, J. L., Dong, Y., Andersen, S. L., Ager Jr., A. L., Fu, H., Miller, R. E., Wesche, D. L., Kyle, D. E., Gerena, L., Walters, S. M., Wood, J. K., Edwards, G., Holme, A. D., McLean, W. G., Milhous, W. K. *J. Med. Chem.* **2000**, *43*, 2753-2758.

<sup>83</sup> Ren, H., Grady, S., Gamenara, D., Heinzen, H., Moyna, P., Croft, S. L., Kendrick, H., Yardley, V., Moyna, G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1851-1854.

<sup>84</sup> Basco, L. K., Dechy-Cabaret, O., Ndounga, M., Meche, F. S., Robert, A., Meunier, B. *Antimicrob. Agents Chemother.* **2001**, *45*, 1886-1888.

aim to combine both pharmacological features. It is expected that the 4-aminoquinoline moiety facilitates the transport to the food vacuole, whereas the trioxane is activated by free Fe<sup>II</sup>-hem liberated during the digestion of hemoglobin. Compound **1.39** (Figure 1.8) is another example of such a hybrid compound. Likewise, chimera of mefloquine and a 10-trifluoromethylartemisinin have been prepared.

The 1,2,4-trioxolane OZ-277 (**1.38**, figure 1.8) is the first synthetic antimalarial endoperoxide which recently entered clinical trials. With the sterically hindrance of an adamantane residue on one and a cyclohexane group on the other side, the critical balance between stability and reactivity could be obtained.<sup>67,85</sup> Addition of an aminoacyl residue further provided the correct polarity and solubility resulting in the desired pharmacological properties.

#### 1.1.7.3. ANTIFOLATES

Tetrahydrofolic acid has a key role in the biosynthesis of thymine, purine nucleotides and several amino acids (Met, Gly, Ser, Glu, His). In contrast to humans, *Plasmodia spp* can synthesize dihydrofolic acid from simple precursors. Dihydropteroate synthase (DHPS) which is absent in humans and dihydrofolate reductase (DHFR) which is sufficiently different from the human enzyme are two important target enzymes in the treatment of malaria (Figure 1.9).

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<sup>85</sup> Dong, Y., Chollet, J., Matile, H., Charman, S. A., Chiu, F. C. K., Charman, W. N., Scorneaux, B., Urwyler, H., Tomas, J. S., Scheurer, C., Snyder, C., Dorn, A., Wang, X., Karle, J. M., Tang, Y., Wittlin, S., Brun, R., Vennerstrom, J. L. *J. Med. Chem.* **2005**, *48*, 4953-4961.

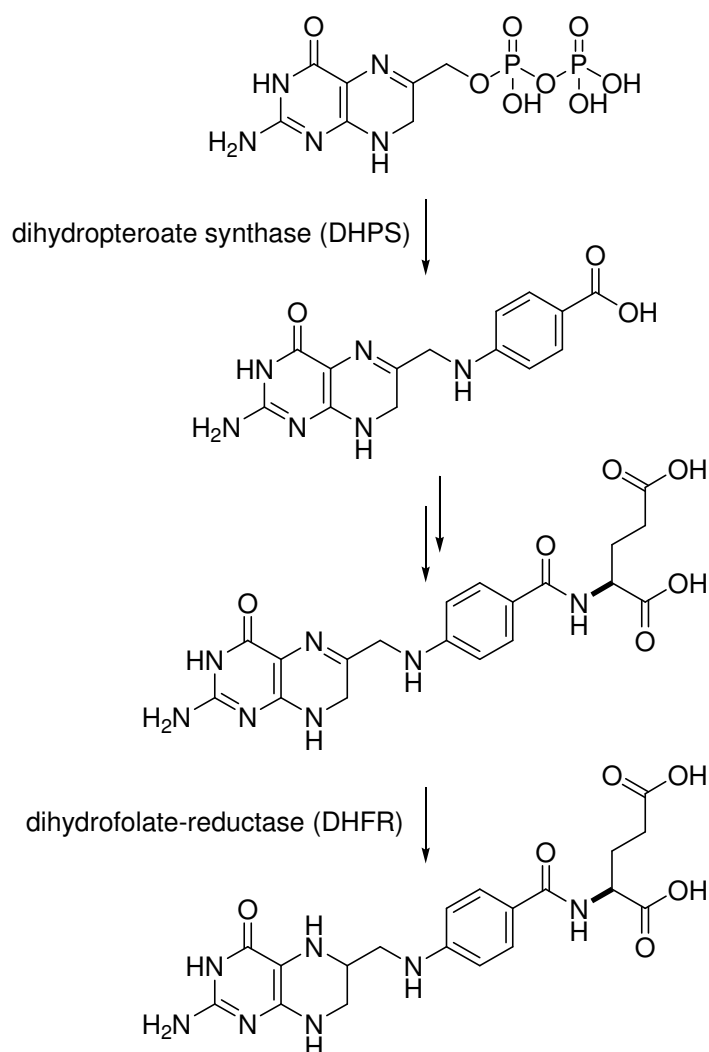


Figure 1.9: Simplified folate pathway showing the targets of the antifolates.

The use of antifolates against malaria<sup>86,87,88,89,90</sup> and the potential of other enzymes of the folate biosynthesis pathway as drug targets have been reviewed.<sup>91,92</sup>

<sup>86</sup> Plowe, C. V. *The History of Antimalarial Drugs, in: Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery* (Ed.: P. J. Rosenthal), Humana Press Inc., Totowa, 173-190.

<sup>87</sup> Gregson, A., Plowe, C. V. *Pharmacol. Rev.* **2005**, *57*, 117-145.

<sup>88</sup> Anderson, A. C. *Drug Discov. Today* **2005**, *10*, 121-128.

<sup>89</sup> Chan, D. C. M., Anderson, A. C. *Curr. Med. Chem.* **2006**, *13*, 377-398.

<sup>90</sup> Nzila, A. *J. Antimicrob. Chemother.* **2006**, *57*, 1043-1054.

<sup>91</sup> Nzila, A., Ward, S. A., Marsh, K., Sims, P. F. G., Hyde, J. E. *Trends Parasitol.* **2005**, *21*, 292-298.

<sup>92</sup> Nzila, A., Ward, S. A., Marsh, K., Sims, P. F. G., Hyde, J. E. *Trends Parasitol.* **2005**, *21*, 334-339.



### 1.1.7.3.1 Established antifolates

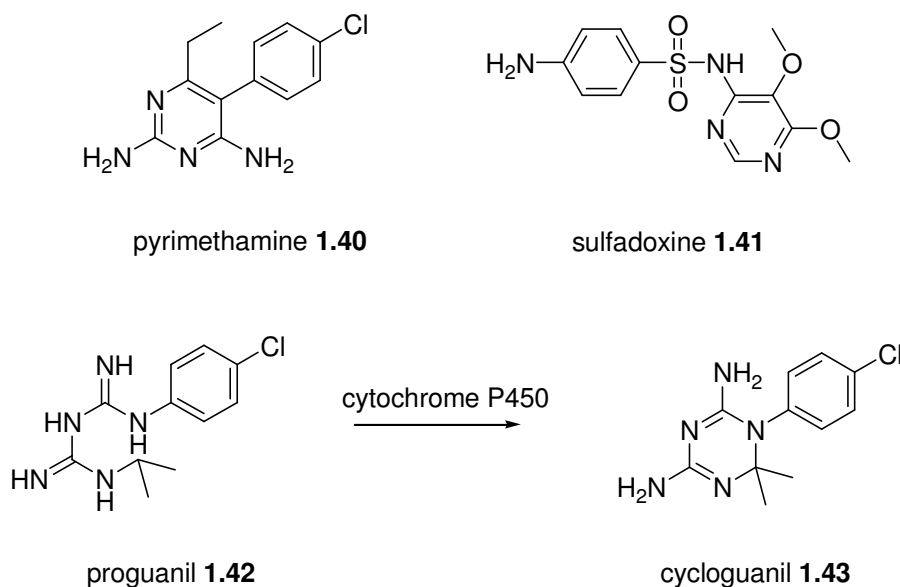


Figure 1.10: Structures of major antifolate drugs.

Pyrimethamine (**1.40**, Figure 1.10) inhibits DHFR. It is mostly combined with the DHPS inhibitor sulfadoxine (**1.41**) to obtain a synergistic effect. The approval of this combination, known as SP, has been retracted in Belgium, because of rapid resistance development and possible side effects. Chloroquine and SP were the two most widely used antimalarial drugs. At an accelerating rate both are failing to produce the desired effect in most malaria endemic regions.<sup>93</sup>

Another important antifolate drug is the prodrug proguanil (**1.42**). Proguanil yields the active metabolite cycloguanil through oxidative ring closure. Cycloguanil (**1.43**) is structurally very similar to pyrimethamine and represents the active form that inhibits DHFR. Proguanil is usually well tolerated and is commonly applied in combination with chloroquine.<sup>94</sup>

Genetic polymorphism by antifolate resistant strains is well-documented. Resistance against DHFR inhibitors developed through consecutive accumulation of mutations in the *dhfr* gene. A key mutation to resistance is the replacement of serine 108 by asparagine (Ser108Asn), which reduces the sensitivity of *Pf*DHFR towards pyrimethamine and cycloguanil 10-fold. Also a triple (Ser108Asn, Asn51Ile and

<sup>93</sup> Greenwood, B., Mutabingwa, T. *Nature* **2002**, 415, 670-672.

<sup>94</sup> Kain, K. C., Shanks, G. D., Keystone, J. S. *Clin. Infect. Dis.* **2001**, 33, 226-234.

Cys59Arg) and a quadruple mutant are currently seen. In parallel to the situation with DHFR the selection of strains which have accumulated several mutations in the *dhps* gene have led to considerable resistance.<sup>95</sup> Although no crystal structure of PfDHPS is available, homology modelling based on the crystal structure of DHPSases of different species have provided some insight in how mutations in the *dhps* gene affect sulfonamide binding.<sup>96,97,98,99</sup> The Ala437Gly and Lys540Glu mutations in the *dhps* gene correlate strongly with resistance. In both enzymes the mutations cause a reduced affinity of the drug for the enzyme.

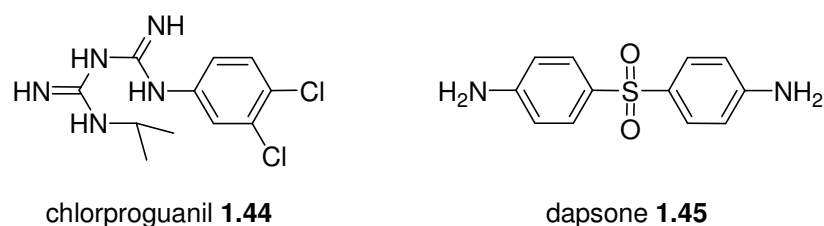


Figure 1.11: Structures of the combination Lap-Dap<sup>®</sup>.

Chlorproguanil-dapsone, known as Lap-Dap<sup>®</sup>, is a combination which has been recently introduced on the market to replace sulfadoxine-pyrimethamine (Figure 1.11).<sup>100</sup> Chlorproguanil (**1.44**) is converted by cytochrome P450 into chlorcycloguanil, acting as a DHFR inhibitor. Dapsone (**1.45**) is a drug used in the treatment of leprosy. Dapsone acts similarly to sulfadoxine as an inhibitor of DHPS. Studies in Africa were very promising, but in South East Asia the combination seemed not to be very efficient, probably due to mutation in the *dhfr* gene.<sup>101</sup> The plasma half-lives of both drugs are low, thereby lowering the chance of resistance, but to decrease the risk of resistance even more a combination of Lap-Dap<sup>®</sup> with

<sup>95</sup> Triglia, T., Wang, P., Sims, P. F. G., Hyde, J. E., Cowman, A. F. *EMBO J.* **1998**, *17*, 3807-3815.

<sup>96</sup> Korsinczky, M., Fischer, K., Chen, N., Baker, J., Rieckmann, K., Cheng, Q. *Antimicrob. Agents Chemother.* **2004**, *48*, 2214-2222.

<sup>97</sup> de Beer, T. A. P., Louw, A. I., Joubert, F. *Bioorg. Med. Chem.* **2006**, *14*, 4433-4443.

<sup>98</sup> Baca, A. M., Sirawaraporn, R., Turley, S., Sirawaraporn, W., Hol, W. G. J. *J. Mol. Biol.* **2000**, *302*, 1193-1212.

<sup>99</sup> Hyde, J. E., Sims, P. F. G. *Trends Parasitol.* **2001**, *17*, 265-266.

<sup>100</sup> Winstanley, P. *Trop. Med. Int. Health.* **2001**, *6*, 952-954.

<sup>101</sup> Wilairatana, P., Kyle, D. E., Looareesuwan, S., Chinwongprom, K., Amradee, S., White, N. J., Watkins, W. M. *Ann. Trop. Med. Parasitol.* **1997**, *91*, 125-132.

artesunate (CDA) is under development.<sup>100</sup> In a phase II clinical study CDA showed significant shorter parasite clearance time than chlorproguanil/dapsone alone.<sup>102</sup>

Lap-Dap<sup>®</sup> is active against strains with the three mutations (Ser108Asn, Asn51Ile and Cys59Arg) in the *dhfr* gene, but concerns have been expressed that the quadruple mutation may be spread when Lap-Dap<sup>®</sup> is extensively used.<sup>103</sup> Cross resistance between several antifolates is also described.

#### 1.1.7.3.2 Novel DHFR inhibitors and other antifolates under development

JPC-2056 (**1.46**) is a prodrug, which is converted via oxidative ring closure to the corresponding dihydrotriazine. It is a promising candidate for pre-clinical development. The dioxypopylene linker allows the adoption of a high affinity conformation while avoiding any repulsive interaction with the Asn108 side chain of DHFR. Cycloguanil derivative (**1.47**) has only one substituent at the 6-position. This modification has been designed to circumvent another mutation (Ala16Val) leading to resistance. Through the move of the chloro substituent to the meta position the critical repulsing interaction by the Asn108 side chain can be avoided.<sup>104</sup> The *m*-bromo derivative (**1.48**) of pyrimethamine inhibits the quadruple mutant DHFR with a  $K_i$  of 5.1 nM.

Trimethoprim (**1.49**) which is widely used in combination with sulfamethoxazole for the treatment of bacterial infections is less active against *Pf*DHFR than pyrimethamine or cycloguanil ( $K_i$  against wild type *Pf*DHFR 10.3 nM). Through replacement of one of the methoxy groups by a benzyloxy group as in **1.50** activity against the wild type DHFR could be markedly improved ( $K_i$  = 0.4 nM). Other structural analogues of known DHFR inhibitors, such as diaminoquinazolines (**1.51**), are reported.

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<sup>102</sup> MMV Press Release, 15<sup>th</sup> November 2005.

<sup>103</sup> Nzila, A., Ochong, E., Nduati, E., Gilbert, K., Winstanley, S. A., Ward, S. A., Marsh, K. *Trans. R. Soc. Trop. Med. Hyg.* **2005**, *99*, 341-346.

<sup>104</sup> McKie, J. H., Douglas, K. T., Chan, C., Roser, S. A., Yates, R., Read, M., Hyde, J. E., Dascombe, M. J., Yuthavong, Y., Sirawaraporn, W. *J. Med. Chem.* **1998**, *41*, 1367-1370.

Compound **1.52** is a precursor of methotrexate that is only converted to the parent drug in parasites. It inhibits the growth of the quadruple resistant strain and proved to be non toxic against human cells.<sup>105</sup>

Resistance against antifolates is not exclusively based on the mutations in the *dhfr* and *dhps* gene described above. In addition to *de novo* synthesis, parasites can also acquire folate from the host via an active transport system. Probenicid (**1.53**) normally used as uricosuricum inhibits this transport system and shows some antifolate resistance reversing properties *in vitro*.<sup>106, 107</sup>

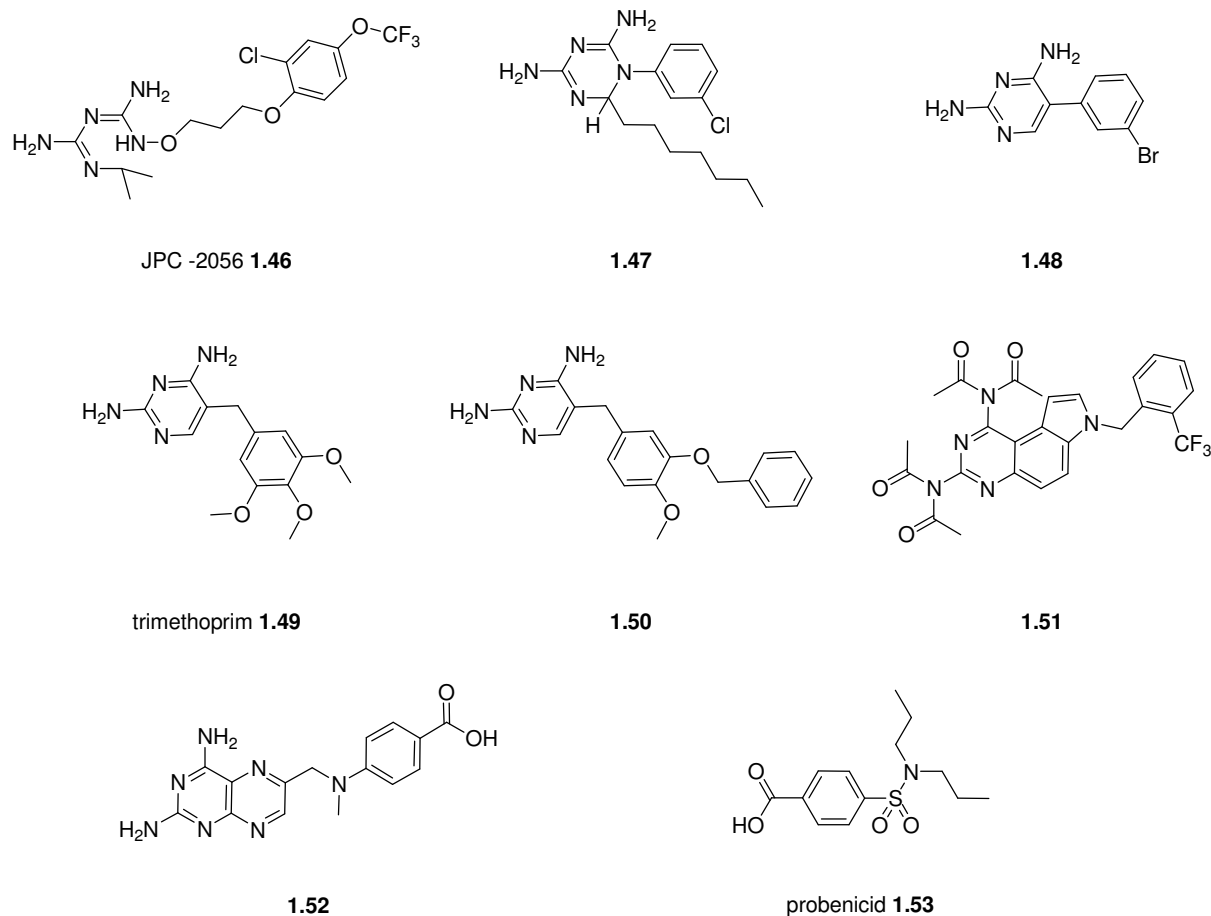


Figure 1.12: Antifolates in development.

<sup>105</sup> Nduati, E., Hunt, S., Kamau, E. M., Nzila, A. *Antimicrob. Agents Chemother.* **2005**, 49, 3652-3657.

<sup>106</sup> Nzila, A. M., Kokwaro, G., Winstanley, P. A., Mash, K., Ward, S. A. *Trends Parasitol.* **2004**, 20, 109-112.

<sup>107</sup> Nzila, A., Mberu, E., Kokwaro, G., Winstanley, P., Mash, K., Ward, S. A. *Antimicrob. Agents Chemother.* **2003**, 47, 2108-2112.

#### 1.1.7.4. ANTIBIOTICS

A variety of antibiotics such as tetracyclines, macrolides, lincosamides, chloramphenicol, spectinomycin, all of which are translation inhibitors in prokaryotic systems, are considered to inhibit protein synthesis inside the apicoplast.<sup>108</sup> Doxycycline (**1.54**, Figure 1.13) is the most frequently used antibiotic in antimalarial therapy, either as a prophylactic agent, or in combination with chloroquine, for treatment of multiresistant malaria.<sup>94</sup> Clindamycin (**1.55**) is a valuable alternative since doxycycline is contra-indicated in small children and pregnant women.<sup>109</sup> Clindamycin binds the 50S subunit of ribosomes in bacteria and inhibits the prokaryote-like protein synthesis machinery of the apicoplast. This results in an arrest of the replication of the organelle.<sup>110</sup>

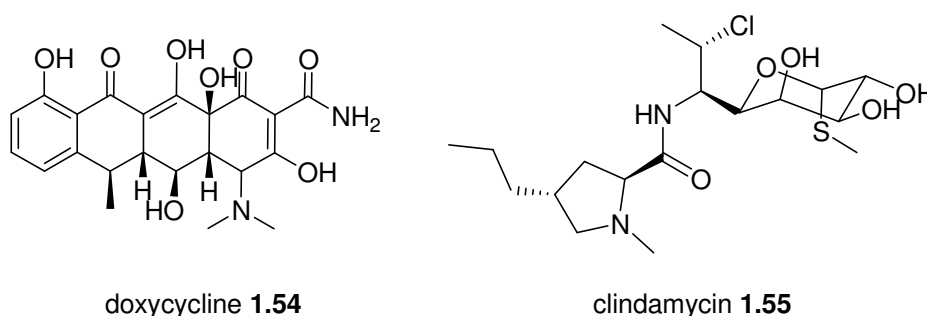


Figure 1.13: Structures of two important antibiotics in the treatment of malaria.

#### 1.1.7.5. COMPOUNDS ACTING ON THE RESPIRATORY CHAIN

Malarone<sup>®</sup> is a fixed combination of atovaquone (**1.56**, Figure 1.14) and proguanil (**1.42**).<sup>111,112</sup> Like its structural analogue ubiquinone (**1.57**), atovaquone inhibits the mitochondrial electron transport by inhibiting the cytochrome c reductase

<sup>108</sup> Clough, B., Wilson, R. J. M. *The History of Antimalarial Drugs*, in: *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery* (Ed.: P. J. Rosenthal), Humana Inc., Totowa, 265-286.

<sup>109</sup> Lell, B., Kremsner, P. G. *Antimicrob. Agents Chemother.* **2002**, 46, 2315-2320.

<sup>110</sup> Fichera, M. E., Roos, D. S. *Nature* **1997**, 390, 407-409.

<sup>111</sup> Looareesuwan, S., Chulay, J. D., Canfield, C. J., Hutchinson, D. B. *Am. J. Trop. Med. Hyg.* **1999**, 60, 533-541.

<sup>112</sup> *Drug Ther. Bull.* **2001**, 39, 73-75.

complex, which causes a collapse of the membrane potential.<sup>113</sup> Remarkably, not cycloguanil but proguanil itself is responsible for this synergistic effect. Proguanil reduces the concentration of atovaquone necessary for the collapse of the membrane potential. The underlying mechanism is unknown.<sup>114,115</sup> Malarone is a usual combination for the prophylaxis against malaria. Especially the activity against liver stages is advantageous as the parasites are killed before an infection of red blood cells can be established.<sup>116</sup> However, the long-term efficacy seems to be limited, due to a documented mutation of the cytochrome gene.<sup>117</sup>

$\beta$ -Methoxyacrylates, which are used as agricultural fungicides, are known to act through a mechanism similar to that of atovaquone. Therefore, a series of  $\beta$ -methoxyacrylate derivatives was screened for antimalarial activity.<sup>118</sup> Compound **1.58** demonstrated the lowest IC<sub>50</sub> value (0.28 nM) ever recorded for an antimalarial drug but rapid development of resistance was observed.<sup>119</sup>

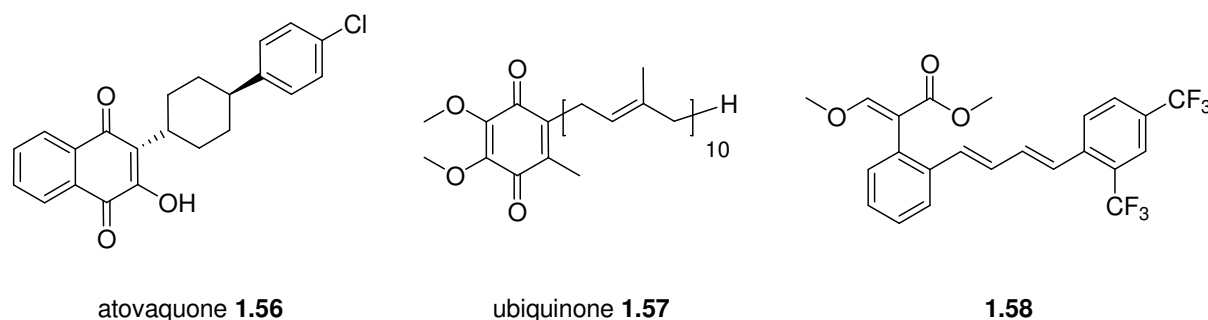


Figure 1.14: Structures of mitochondrial electron transport inhibitors.

#### 1.1.7.6. PROTEASE INHIBITORS

Hemoglobine is consumed in the food vacuole of the *P. falciparum* parasite during its intra-erythrocytic stages. A lot of specific proteases are involved in this

<sup>113</sup> Fry M., Pudney M. *Biochem. Pharmacol.* **1992**, 43, 1545-1553.

<sup>114</sup> Canfield, C. J., Pudney, M., Gutteridge, W. E. *Exp. Parasitol.* **1995**, 80, 373-381.

<sup>115</sup> Srivastava, I. K., Vaidya, A. B. *Antimicrob. Agents Chemother.* **1999**, 43, 1334-1339.

<sup>116</sup> Berman, J. D., Nielsen, R., Chulay, J. D., Dowler, M., Kain, K. C., Kester, K. E., Williams, J., Whelen, A. C., Shmuklarsky, M. J. *Trans. R. Soc. Trop. Med. Hyg.* **2001**, 95, 429-432.

<sup>117</sup> Fivelman, Q. L., Butcher, G. A., Adagu, I. S., Warhurst, D. C., Pasvol, G. *Malar. J.* **2002**, 1, 1.

<sup>118</sup> Alzeer, J., Chollet, J., Heinze-Krauss, I., Hubschwerlen, C., Matile, H., Ridley, R. G. *J. Med. Chem.* **2000**, 43, 560-568.

<sup>119</sup> Ridley, R. G. *Nature* **2002**, 415, 686-693.

proces, such as the aspartoproteases plasmepsin I, II and IV<sup>120,121</sup> and the cysteine proteases falcipain-2 and 3. Inhibitors of these well-characterized enzymes are potential new drug candidates. Potential inhibitors were designed through modification of available inhibitors of cathepsin D (a human aspartoprotease), such as compound **1.59**,<sup>122</sup> and by using the crystal structure of plasmepsin II, for *de novo* design of inhibitors, such as compound **1.60** (Figure 1.15).<sup>123</sup> Some compounds, such as Ro 42-1118 (**1.61**) were originally described as plasmepsin II inhibitors, but exhibited a 16 to 80-fold higher potency against plasmepsin IV.<sup>121</sup> Compounds **1.62** and **1.63** are cysteine protease inhibitors and showed promising IC<sub>50</sub> values (respectively 4 and 0.4 nM) against *in vitro* *P. falciparum* growth.

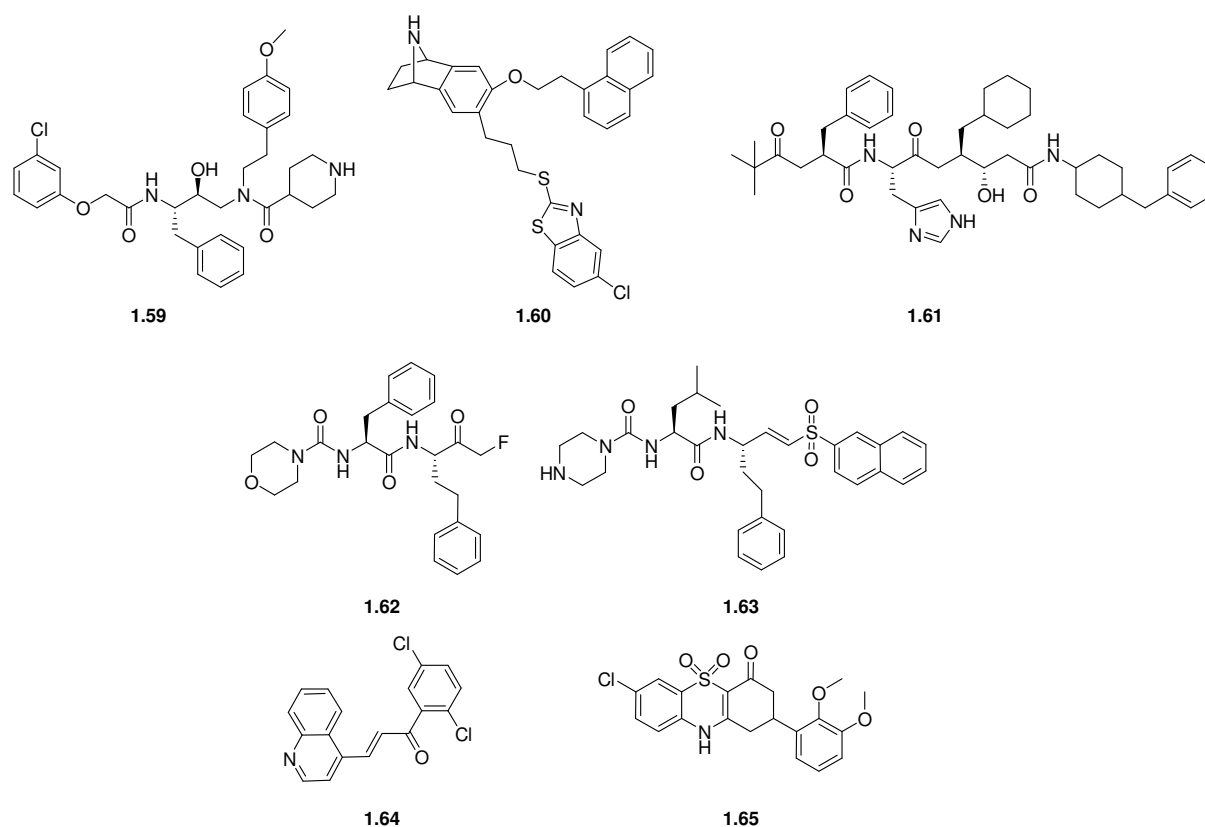


Figure 1.15: Structures of protease inhibitors.

<sup>120</sup> Coombs, G. H., Goldberg, D. E., Klemba, M., Berry, C., Kay, J., Mottram, J. C. *Trends Parasitol.* **2001**, 17, 532-537.

<sup>121</sup> Wyatt, D. M., Berry, C. *FEBS Lett.* **2002**, 513, 159-162.

<sup>122</sup> Haque, T. S., Skillman, A. G., Lee, C. E., Habashita, H., Gluzman, I. Y., Ewing, T. J., Goldberg, D. E., Kuntz, I. D., Ellman, J. A. *J. Med. Chem.* **1999**, 42, 1428-1440.

<sup>123</sup> Carcache, D. A., Hörtner, S. R., Bertogg, A., Binkert, C., Bur, D., Märki, H. P., Dorn, A., Diederich, F. *ChemBioChem* **2002**, 3, 1137-1141.

Attempts to develop non-peptide falcipain-2 inhibitors led to the identification of chalcones (e.g., **1.64**) and phenothiazine like compounds (e.g., **1.65**). Compound **1.64** is the chalcone with the highest reported *in vitro* activity ( $IC_{50} = 230$  nM).<sup>124</sup>

#### 1.1.7.7. INHIBITORS OF FATTY ACID SYNTHESIS

The presence of a fatty acid biosynthesis pathway in the apicoplast (*vide infra* paragraph 1.2.3.) of *P. falciparum* has recently been established (Figure 1.3). This pathway represents the so-called “type II system” (FAS-II)<sup>125</sup> common to plants and bacteria, but distinct from the so-called “type I system” (FAS-I)<sup>126</sup> of animals including humans. Thiolactomycin (**1.66**, Figure 1.16), a natural antibiotic, specifically inhibits the FAS-II system and shows *in vitro* growth inhibition of *P. falciparum* ( $IC_{50} = 50$   $\mu$ M).<sup>125</sup> Triclosan (**1.67**), known as preservative in toothpastes, is a significantly more active ( $IC_{50} \approx 1$   $\mu$ M against FabI, an enzyme involved in FASII),<sup>127</sup> but is not suitable for oral use.

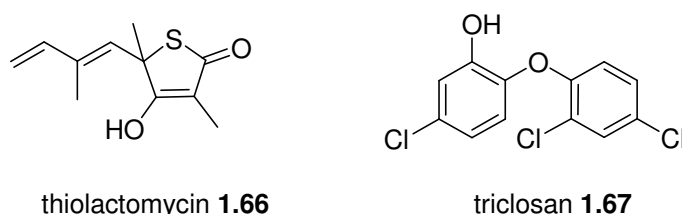


Figure 1.16: Structures of inhibitors of the *P. falciparum* fatty acid biosynthesis.

#### 1.1.7.8. INHIBITORS OF PHOSPHOLIPID METABOLISM

The malaria parasite needs big amounts of phospholipids such as phosphatidylcholine for the synthesis of new membranes during the intra-erythrocytic

<sup>124</sup> Li, R., Kenyon, G. L., Cohen, F. E., Chen, X., Gong, B., Dominguez, J. N., Davidson, E., Kurzban, G., Miller, R. E., Nuzum, E. O., Rosenthal, P. J., McKerrow, J. H. *J. Med. Chem.* **1995**, *38*, 5031-5037.

<sup>125</sup> Waller, R. F., Keeling, P. J., Donald, R. G., Striepen, B., Handman, E., Lang-Unnasch, N., Cowman, A. F., Besra, G. S., Roos, D. S., McFadden, G. I. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12352-12357.

<sup>126</sup> Heath, R. J., White, S. W., Rock, C. O. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 695-703.

<sup>127</sup> Surolia, N., Surolia, A. *Nat. Med.* **2001**, *7*, 167-173.



development. The phospholipid metabolism as a target for antimalarial drugs has been reviewed.<sup>128, 129</sup> Compound G25 (**1.68**, Figure 1.17) emerged as a lead from the first generation of bis quaternary ammonium compounds. It inhibits the growth of cultured parasites with an  $IC_{50}$  value of 0.64 nM. Due to toxic side effects and the lack of oral bioavailability, a second generation was developed in which the quaternary ammonium groups were replaced by bioisosteric amidino and guanidino groups. However, compound MS1 (**1.69**), a lead of the second generation, still carries permanent charges. MS1 displays high *in vitro* activity ( $IC_{50}$  = 0.3 nM), but the desired oral activity could not be obtained.

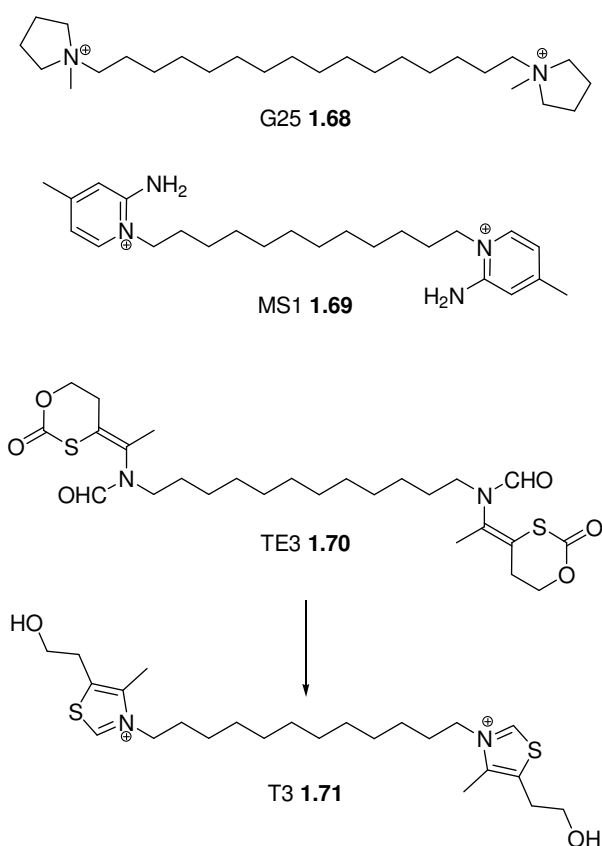


Figure 1.17: Structures of inhibitors of phospholipid metabolism.

Success came with a third generation of bis-thiazolium compounds. Compound TE3 (**1.70**) represents a thioester prodrug which is rapidly cleaved and

<sup>128</sup> Salom-Roig, X. J., Hamzé, A., Calas, M., Vial, H. J. *Comb. Chem. High Throughput Screen.* **2005**, *8*, 49-62.

<sup>129</sup> Vial, H. J., Calas, M. *The History of Antimalarial Drugs, in: Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery* (Ed.: P. J. Rosenthal), Humana Press Inc., Totowa, 347-365.

rearranged in blood to the bis-thiazolium compound T3 (**1.71**,  $IC_{50} = 2.25$  nM). Bis-ammonium compounds cross the infected erythrocyte by parasite induced new permeability pathways (NPP) and enters the parasite using a choline transporter. Inside the parasites the phosphatidylcholine *de novo* synthesis is inhibited either through the inhibition of the choline uptake or the inhibition of enzymes of this pathway or the combination of both effects.<sup>130</sup> The bis-ammonium compounds can be regarded as dual drugs since they have shown to bind also to FPIX.

#### 1.1.7.9. INHIBITORS OF FARNESYL TRANSFERASE

Farnesyl transferase inhibitors are known from anticancer therapy, because the farnesylation of proteins is important for signal transduction. Protein farnesylation has also been demonstrated in *Plasmodium*.<sup>131</sup> The reaction catalyzed by farnesyl transferase is depicted in Figure 1.18.

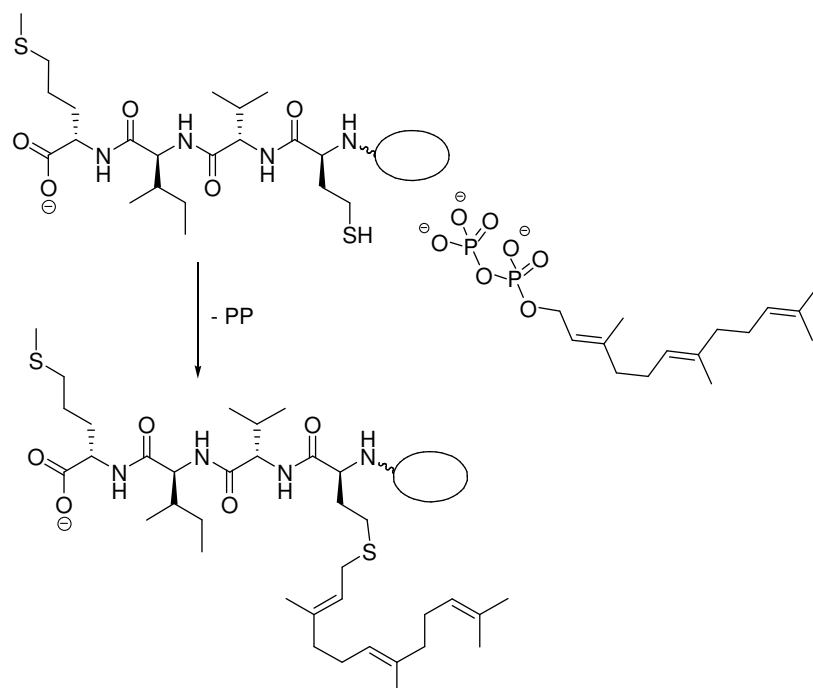


Figure 1.18: Farnesyl transferase catalyzed reaction.

<sup>130</sup> Roggero, R., Zufferey, R., Minca, M., Richier, E., Calas, M., Vial, H., Mamoun, C. B. *Antimicrob. Agents Chemother.* **2004**, *48*, 2816-2824.

<sup>131</sup> Chakrabarti, D., Azam, T., DelVecchio, C., Qiu, L., Park, Y. L., Allen, C. M. *Mol. Biochem. Parasitol.* **1998**, *94*, 175-184.

Farnesyl transferase is a heterodimeric zinc protein, which catalyzes the transfer of a farnesyl moiety, from farnesylpyrophosphate to the carboxyterminal CaaX-recognition sequence of proteins (C = cysteine, a = amino acid with aliphatic side chain, X = serine or methionine). Most inhibitors are thiol free Caax peptide mimetics. Among the most active farnesyl transferase inhibitors (Figure 1.19) are the biphenyl derivative FTI-2135 (**1.72**, *in vitro* antimalarial activity  $IC_{50} = 4.4 \mu M$ ) and the benzophenone-based compound Schl-4116 (**1.73**, *in vitro* antimalarial activity  $IC_{50} = 75 \text{ nM}$ ).<sup>132,133</sup> Despite the high sequence homologies within the active sites of human and parasitic farnesyl transferases, the design of specific inhibitors appears feasible, as demonstrated by the Schl-4116 compound.<sup>134</sup>

Bisphosphonates (Figure 1.19), used in the treatment of osteoporosis, are assumed to inhibit farnesyl pyrophosphate synthase.<sup>135</sup> Compounds **1.74** and **1.75** appeared active *in vitro* ( $IC_{50}$  respectively 5.1 and 7.7  $\mu M$ ).

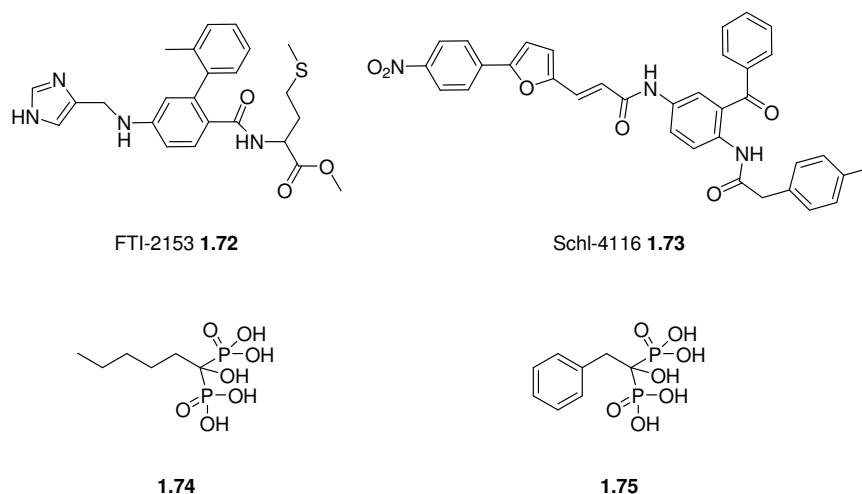


Figure 1.19: Structures of farnesyl transferase inhibitors (**1.72-1.73**) and farnesyl pyrophosphate synthase inhibitors (**1.74-1.75**).

<sup>132</sup> Okhanda, J., Lockman, J. W., Yokoyama, K., Gelb, M. H., Croft, S. L., Kendrick, H., Harrell, M. I., Feagin, J. E., Blaskovich, M. A., Sehti, S. M., Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 761-764.

<sup>133</sup> Wiesner, J., Mitsch, A., Wißner, P., Krämer, O., Jomaa, H., Schlitzer, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2681-2683.

<sup>134</sup> Wiesner, J., Ortmann, R., Mitsch, A., Wißner, P., Sattler, I., Jomaa, H., Schlitzer, M. *Pharmazie* **2003**, *58*, 289-290.

<sup>135</sup> (a) Martin, M. B., Grimley, J. S., Lewis, J. C., Heath III, H. T., Bailey, B. N., Kendrick, H., Yardley, V., Caldera, A., Lira, R., Urbina, J. A., Moreno, S. N., Docampo, R., Croft, S. L., Oldfield, E. *J. Med. Chem.* **2001**, *44*, 909-916. (b) Leon, A., Liu, L., Yang, Y., Hudock, M. P., Hall, P., Yin, F., Studer, D., Puan, K.-J., Morita, C. T., Oldfield, E. *J. Med. Chem.* **2006**, *49*, 7331-7341.

#### 1.1.7.10. INHIBITORS OF GLYCOLYSIS

Some enzymes involved in the glycolytic pathway of *P. falciparum*, such as lactate dehydrogenase, are significantly different from their human isozymes and are well characterized.<sup>136</sup> High-throughput screening and structure-based design may afford specific inhibitors. The malaria parasite exclusively depends on glycolysis for its energy supply, since a Krebs cycle is absent.<sup>137</sup> The structures of other glycolytic enzymes, such as fructose-1,6-bisphosphate aldolase and triosephosphate isomerase have been solved and afford new attractive targets for drug design.<sup>138,139</sup>

#### 1.1.7.11. OTHER ANTIMALARIAL COMPOUNDS

A number of reviews discuss new targets and lead structures for malaria.<sup>140,141,142,143</sup> In Figure 1.20 some recent lead structures that can act as platform for the design of new antimalarial drugs are displayed. WR182393 (**1.76**) is an analogue of chlorproguanil that cannot be cyclised. It is only active against the liver stages and shows a different mechanism of action.<sup>144</sup>

Dioncophyllines such as **1.77** are isolated from tropical lianas that are applied in traditional medicine in African and Asian countries.<sup>145</sup> Febrifugine (**1.78**), an active ingredient from the Chinese plant *Dichora febrifuga*, has proven antimalarial activity, both *in vivo* as *in vitro*, but shows some severe adverse effects. Its metabolite, 3''-ketofebrifugine (**1.79**), appeared to be less toxic and was slightly more active than chloroquine.<sup>146</sup> The mode of action of the dioncophyllines and the febrifugines is unknown to date.

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<sup>136</sup> Dunn, C. R., Banfield, M. J., Barker, J. J., Higham, C. W., Moreton, K. M., Turgut-Balik, D., Brady, R. L., Holbrook, J. J. *Nat. Struct. Biol.* **1996**, 3, 912-915.

<sup>137</sup> Sherman, I. W. *Microbiol. Rev.* **1979**, 43, 453-495.

<sup>138</sup> Velanker, S. S., Ray, S. S., Gokhale, R. S., Suma, S., Balaram, H., Balaram, P., Murthy, M. R. *Structure* **1997**, 5, 751-761.

<sup>139</sup> Kim, H., Certa, U., Dobeli, H., Jakob, P., Hol, W. G. *Biochemistry* **1998**, 37, 4388-4396.

<sup>140</sup> Olliaro, P. L., Yuthavong, Y. *Pharmacol. Ther.* **1999**, 81, 91-110.

<sup>141</sup> Gutteridge, W. E. *Parassitologia* **1999**, 41, 449-452.

<sup>142</sup> Chauhan, P. M., Srivastava, S. K. *Curr. Med. Chem.* **2001**, 8, 1535-1542.

<sup>143</sup> Gelb, M. H., Hol, W. G. *Science* **2002**, 297, 343-344.

<sup>144</sup> Milhous, W. K. *Med. Trop.* **2001**, 61, 48-50.

<sup>145</sup> Francois, G., Timperman, G., Eling, W., Assi, L. A., Holenz, J., Bringmann, G. *Antimicrob. Agents Chemother.* **1997**, 41, 2533-2539.

<sup>146</sup> Kikuchi, H., Tasaka, H., Hirai, S., Takaya, Y., Iwabuchi, Y., Ooi, H., Hatakeyama, S., Kim, H. S., Wataya, Y., Oshima, Y. *J. Med. Chem.* **2002**, 45, 2563-2570.

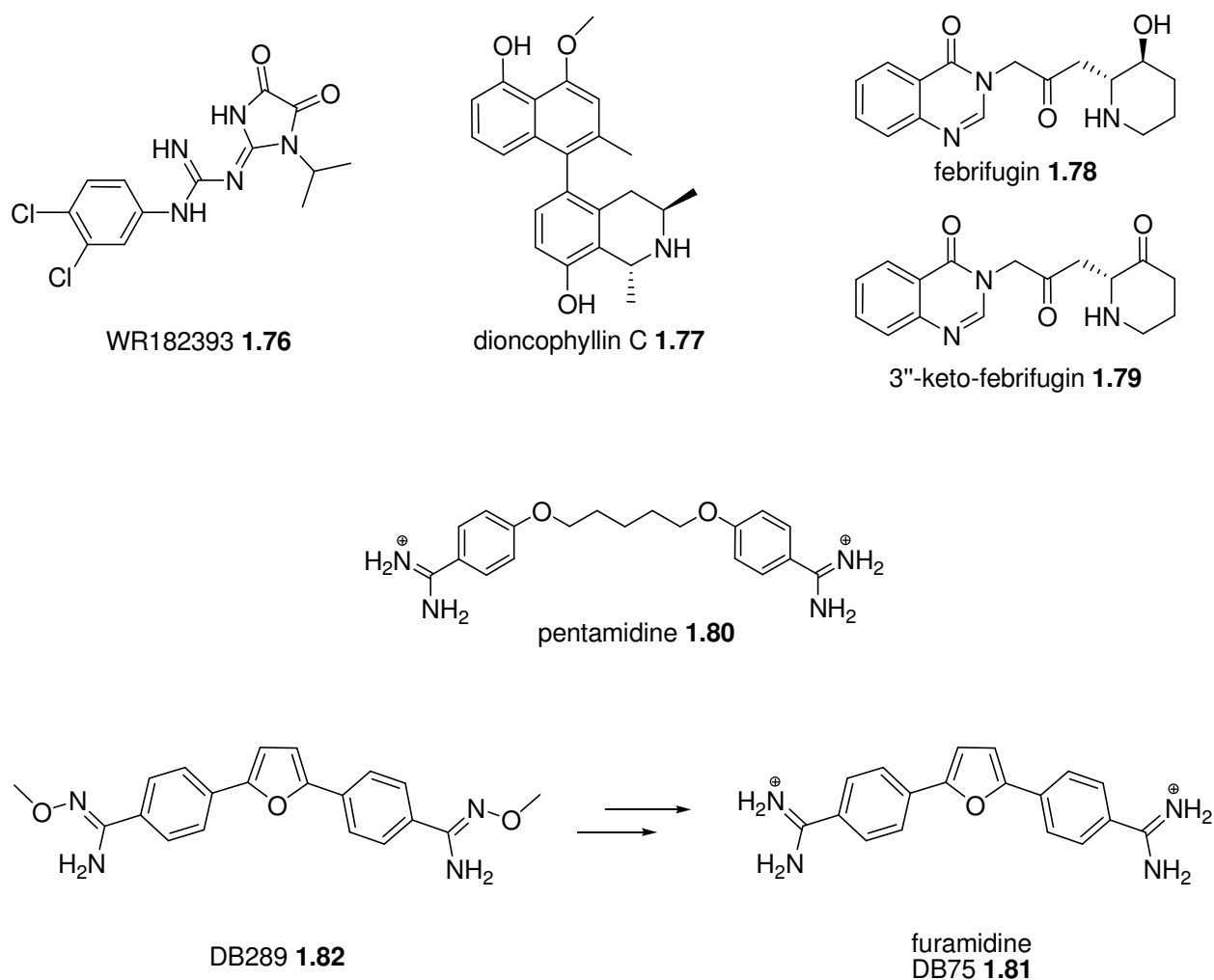


Figure 1.20: Other new antimalarial compounds.

Diamidines have a long history as antiprotozoal agents. Today, pentamidine (**1.80**) is the only diamidine in clinical use for trypanosomiasis. Diamidines enter the infected erythrocyte through new permeability pathways (NPP) and the parasite through the choline carrier. Recently, it has been proposed that diamidines act against *Plasmodium* through the FPIX-binding (see 1.1.7.1) and the inhibition of mitochondrial functions.<sup>147</sup> Furamidine (**1.81**) (DB75) in which the pentamidine flexible chain has been replaced by a rigid 2,5-substituted furyl residue is the most advanced compound.<sup>148</sup> Because these compounds are insufficiently absorbed from the gastrointestinal tract after oral administration, *O*-methylamidoxim-prodrugs are

<sup>147</sup> Werbovets, K. *Curr. Opin. Invest. Drugs* **2006**, 7, 147-157.

<sup>148</sup> Yeates, C. *IDrugs* **2003**, 6, 1086-1093.

made. DB289 (**1.82**) is in phase II clinical trials against *P. vivax* and uncomplicated *P. falciparum* infections. This trial demonstrated high efficiency and good tolerability for this drug.<sup>149</sup> Several diamidines are under preclinical development. The high *in vitro* activity against multi-resistant strains, the oral bioavailability of alkylamidoxim prodrugs and the efficiency and tolerability of DB289 in a first clinical trial suggest that the diamidines constitute a highly promising class of antimalarials.

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<sup>149</sup> Yeramian, P., Meshnick, S. R., Krudsood, S., Chalermrut, K., Silachamroon, U., Tangpukdee, N., Allen, J., Brun, R., Kwiek, J. J., Tidwell, R., Looareesuwan, S. *J. Infect. Dis.* **2005**, *192*, 319-322.

## **1.2. THE NON-MEVALONATE PATHWAY AS DRUG TARGET**

### **1.2.1. DRUG DISCOVERY AND THE ISOPRENOID BIOSYNTHESIS**

Due to the increasing resistance against conventional anti-malarial drugs, there is an urgent need for new efficient and safe treatments. The discovery of novel therapeutics largely relies on four strategies<sup>150</sup>:

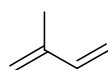
- modification of existing anti-parasitic drugs into improved drugs with higher activity, less side effects or (and) more favourable pharmacokinetic properties, label extension is another possibility,
- the “piggy back” strategy: targets being pursued for other indications but present in parasites will facilitate the identification of new lead compounds. These leads can be further developed into selective “drugable” compounds,
- “de novo” drug discovery: new hits can be found by target based screening of chemical libraries for a specific biological activity in whole parasite assays or against a specific protein and subsequently developed.
- another “de novo” strategy relies on the investigation of parasite selective biochemical pathways affording information on the drug target properties of these pathways. Inhibitors of such a pathway can be discovered by screening procedures or through rational drug design strategies.

The latter approach is particularly attractive in the discovery of new chemotherapeutic agents. Pathogenic micro-organisms often possess specific biochemical pathways which are essential for their survival and do not occur in mammals. Inhibitors of these pathways may show antiparasitic activity combined with low toxicity in humans.

The “non-mevalonate pathway” shows excellent target properties for antimalarial drug discovery. This pathway is responsible for the biosynthesis of dimethylallyl pyrophosphate (DMAPP) and its isomer isopentenyl pyrophosphate (IPP) in plasmodia. Both pyrophosphates are the building blocks for an impressive group of biomolecules, the isoprenoids. Since we selected the “non-mevalonate pathway” as a target to design new antimalarials, this pathway will be discussed in detail.

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<sup>150</sup> Nwaka, S., Hudson, A. *Nat. Rev. Drug Discovery* **2006**, 5, 941-955.



isoprene

Figure 1.21: Structure of isoprene.

Isoprenoids are natural products and polymers composed of isoprene (2-methyl-1,3-butadiene, Figure 1.21) units. Two  $C_5$  units condense to form geranyl pyrophosphate ( $C_{10}$ ), which condenses with another molecule of IPP to form farnesyl pyrophosphate ( $C_{15}$ ). In humans these pyrophosphates are important for the biosynthesis of steroids, vitamin D, bile salts, cholesterol, etc. In plants they are crucial building blocks for primary and secondary metabolites: the terpenes ( $C_5$  and  $C_{15}$  compounds), squalene (an assembly of two  $C_{15}$  units) and carotenoids (an assembly of  $C_{20}$  units), chlorophylls and plastoquinones. Isoprenoids such as sterols, ubiquinones, dolichols and carotenoids are essential for all organisms. Today there are over 30000 structures known with an isoprenoid substructure.

### **1.2.2. THE MEVALONATE VERSUS THE NON-MEVALONATE PATHWAY**

In animals (including humans), fungi, archaeobacteria and some eubacteria, isoprenoids are synthesized by the mevalonate pathway, which has been well-characterized since the 1960s (Figure 1.22). The mevalonate pathway starts with the condensation of three molecules of acetyl coenzyme A (acetyl CoA) into 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), which is reduced to mevalonate by HMG-CoA reductase. Mevalonate is converted by phosphorylation and an elimination step into isopentenyl pyrophosphate (IPP).<sup>151</sup>

Until the 1980s it was thought that the mevalonate pathway was the only biosynthetic avenue to isoprenoids, but labelling studies conflicted with this hypothesis. The incorporation of labelled acetate, glucose and pyruvate in bacterial

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<sup>151</sup> Beytia, E. D., Porter, J. W. *Annu. Rev. Biochem.* **1976**, 45, 113-142.



terpenoids gave unexpected patterns.<sup>152</sup> Surprising labelling patterns were also obtained by incorporation of <sup>13</sup>C-labelled glucose in ubiquinones in *Escherichia coli* and ginkgolides in *Ginkgo biloba*. A mechanism starting with the condensation of glyceraldehyde 3-phosphate and pyruvate was proposed.<sup>153,154</sup> The resulting 1-deoxy-D-xylulose 5-phosphate (DOXP) was incorporated into the prenyl chains of ubiquinone from *E. coli*. At the same time the research group of Rohmer elucidated a different pathway for isoprenoid biosynthesis.<sup>155,156</sup> These reports were the first to show the existence of an alternative mevalonate-independent pathway for isoprenoid biosynthesis in plants and bacteria. This mevalonate-independent pathway is also called non-mevalonate pathway, DOXP pathway, Rohmer pathway or 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Figure 1.22).

The DOXP pathway is absent in humans and fungi, but present in many eubacteria and some protozoa.<sup>157,158</sup> In some eubacteria, however, a functional DOXP and a mevalonate pathway co-exist.<sup>159</sup> In plants, both pathways are present with the DOXP pathway being operative in the plastids and the mevalonate pathway in the cytosol.<sup>160</sup> Since it is essential for survival of the parasites and absent in humans, the DOXP pathway represents a promising target for developing novel anti-infectious agents

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<sup>152</sup> Flesch, G., Rohmer, M. *Eur. J. Biochem.* **1988**, *175*, 405-411.

<sup>153</sup> Broers, S. T. J. *PhD Thesis* **1994**, EHT Zürich, Switzerland.

<sup>154</sup> Schwarz, M. K. *PhD Thesis* **1994**, EHT Zürich, Switzerland.

<sup>155</sup> Romher, M., Knani, M., Simonin, P., Sutter, B., Sahm, H. *Biochem J* **1993**, *295*, 517-524.

<sup>156</sup> Romher, M. *Nat. Prod. Rep.* **1999**, *16*, 565-574.

<sup>157</sup> Boucher, Y., Ford Doolittle, W. *Mol. Microbiol.* **2000**, *37*, 703-716.

<sup>158</sup> Rodríguez-Concepción, M. *Curr. Pharm. Design* **2004**, *10*, 2391-2400.

<sup>159</sup> Seto, H., Watanabe, H., Furihata, K. *Tetrahedron Lett* **1996**, *37*, 7979-7982.

<sup>160</sup> Lichtenthaler, H. K., Rohmer, M., Schwender, J. *Physiol Plant* **1997**, *101*, 643-652.

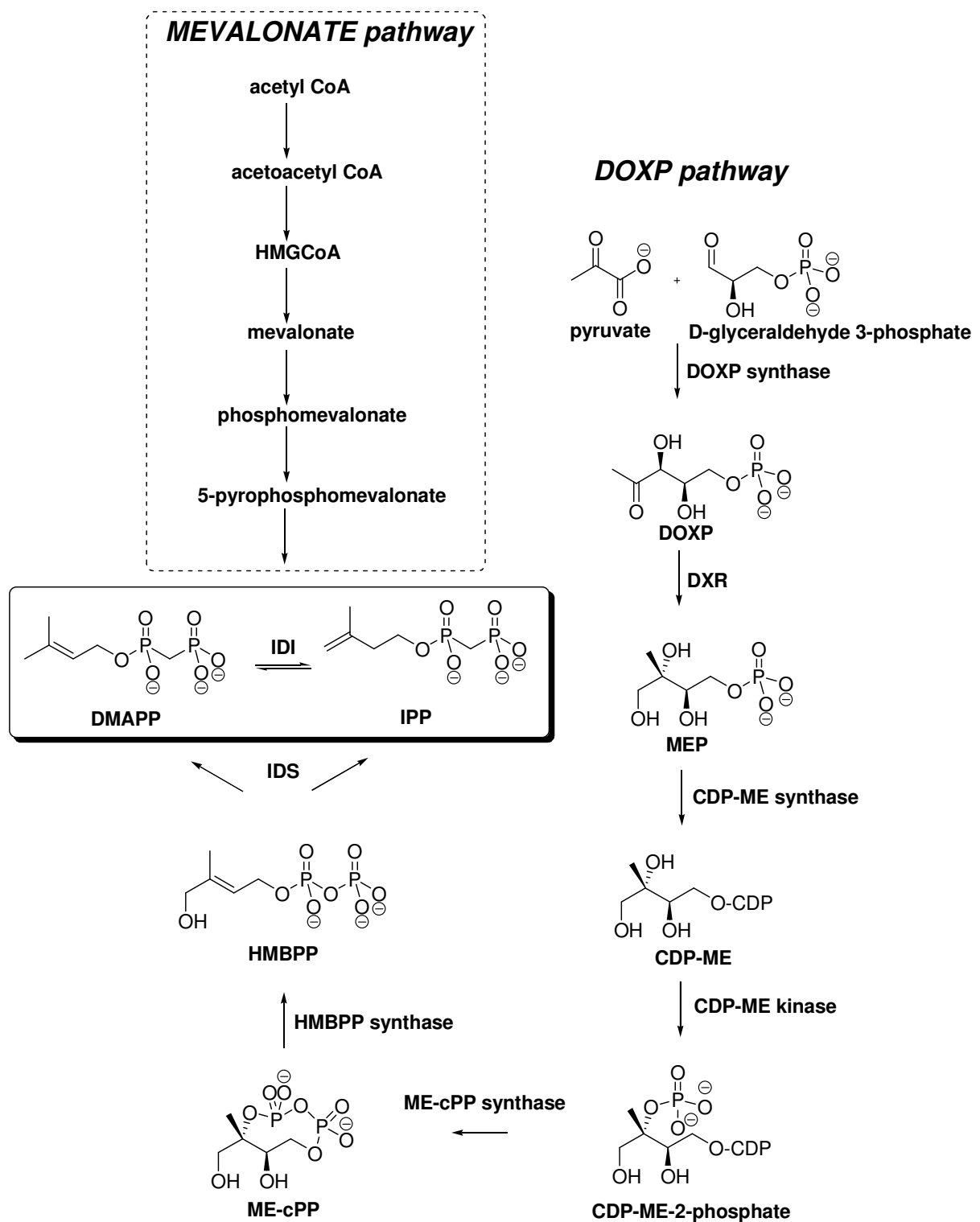


Figure 1.22: Overview of the isoprenoid biosynthesis via the mevalonate and the DOXP pathway (CDP = diphosphocytidyl).

Figure 1.22 depicts the DOXP pathway. Investigation of this pathway was mainly performed on *E.coli* and various plants. The enzymes involved have been cloned from a variety of higher plants, bacteria and protozoa.<sup>161</sup>

The first reaction of the DOXP pathway is the condensation of D-glyceraldehyde 3-phosphate and pyruvate by means of DOXP synthase (DXS). The gene of DXS was discovered independently by several research groups.<sup>162,163,164</sup> DOXP was previously known as a precursor for thiamine diphosphate and pyridoxol phosphate in *E. coli*.

In the second step, DOXP is converted into the branched-chain polyol 2-C-methyl-D-erythritol 4-phosphate (MEP) by DOXP reductoisomerase (DXR, IspC). This enzyme catalyzes a reversible rearrangement and a NADPH-dependent reduction.<sup>165,166</sup> Currently, DXR is clinically the most relevant and also the best studied enzyme of the DOXP pathway.

In the next step 4-diphosphocytidyl-2-C-methyl-D-erythritol (CDP-ME) is formed by a nucleotidylation with CDP-ME synthase (IspD, YgbP) and CTP as cofactor. It is subsequently phosphorylated at the 2-position by CDP-ME kinase (CMK, IspE) with the formation of CDP-ME-2-phosphate. The latter is cyclized by ME-cPP synthase (IspF, YgbB) into 2-C-methyl-D-erythritol-2,4-cyclodiphosphate (ME-cPP) and subsequently transformed to 2-hydroxymethyl-*cis*-but-2-en-4-yl diphosphate (HMBPP) by HMBPP synthase (GcpE, IspG). In the last step, HMBPP is converted to IPP or DMAPP. Both reactions can be catalyzed by IPP/DMAPP synthase (IDS, LytB, IspH). In contrast to organisms that rely on the mevalonate pathway, organisms using the DOXP pathway are not dependent on isopentenyl diphosphate isomerase (IDI), which catalyzes the isomerisation of IPP into DMAPP.

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<sup>161</sup> Eisenreich, W., Bacher, A., Arigoni, A., Rohdich, F. *Cell. Mol. Life Sci.* **2004**, *61*, 1401-1426.

<sup>162</sup> Sprenger, G. A., Schorken, U., Wiegert, T., Grolle, S., De Graaf, A. A., Rohdich, F. *J. Biol. Chem.* **1997**, *94*, 12857-12862.

<sup>163</sup> Lange, B. M., Wildung, M. R., McCaskill, D., D. Croteau, R. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2100-2104.

<sup>164</sup> Lois, L. M., Campos, N., Rosa Putra, S., Danielsen, K., Rohmer, M., Boronat, A. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2105-2110.

<sup>165</sup> Takahashi, S., Kuzuyama, T., Watanabe, W., Seto, H. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9879-9884.

<sup>166</sup> Koppisch, A. T., Fox, D. T., Blagg, S. J. B., Poulter, C. D. *Biochemistry* **2002**, *41*, 236-243.

### 1.2.3. DOXP PATHWAY IN *P. FALCIPARUM*

Previous studies revealed very low HMG-CoA reductase activity in *P. falciparum*.<sup>167</sup> Attempts to establish HMG-CoA reductase inhibitors as antimalarial drugs failed,<sup>168</sup> suggesting the absence of a mevalonate pathway in *P. falciparum*. In 1999 Jomaa and coworkers found that *P. falciparum* relies on the DOXP pathway, where it is localized inside a plastid-like organelle, the apicoplast. By searching for genes encoding enzymes of the DOXP pathway, similarities were found between known bacterial sequences of DXR and sequences in the genome of *P. falciparum*.

In general, the genes encoding for DXR show a great degree of similarity among different species. Nevertheless, if the alignment of the amino acid sequence between *E. coli*, *Bacillus subtilis*, *Synechocystis* and *P. falciparum* is compared, DXR of *P. falciparum* has a unique amino-terminal extension. This extension exists of approximately 70 amino acids, from which the first 30 amino acids are part of an endoplasmic reticulum signal peptide and the last 44 amino acids form a typical plastidial targeting sequence. This extra polypeptide chain implies that DXR, once it is formed, finds its way to the apicoplast.

If the gene of this targeting sequence is fused to the gene of green fluorescent protein (GFP) and these genes are brought to expression by transfected *Toxoplasma gondii*, it can be visualized that GFP accumulates in the apicoplast. In the apicoplast of *Apicomplexa*, as well as in the chloroplast of plants, isoprenoids are synthesized via the DOXP pathway. Also, the synthesis of fatty acids and, potentially hem, take place in the apicoplast. The apicoplast has been acquired during the evolution by endosymbiosis with an alga, which subsequently has lost its photosynthetic activity.

The apicoplast contains an independent genome. This genome is responsible only for the self-reproduction of the apicoplast. The genes for the isoprenoid biosynthesis are part of the nuclear DNA. Thus, the enzymes involved in this biosynthesis inside the apicoplast are all encoded by the nuclear genome, synthesized in the cytosol and transported to the apicoplast. Therefore, these enzymes have an apicoplast-targeting sequence.

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<sup>167</sup> Vial, H. J., Philpott, J. R., Wallach, D. F. *Mol. Biochem. Parasitol.* **1984**, 13, 53-65.

<sup>168</sup> Grellier, P., Valentin, A., Millerioux, V., Schrevel, J., Rigomier, D. *Antimicrob. Agents Chemother.* **1994**, 38, 1144-1148.

## 1.2.4. FOSMIDOMYCIN, AN INHIBITOR OF THE DOXP PATHWAY

### 1.2.4.1. DISCOVERY AND ANTIBACTERIAL ACTIVITY

During the 1970s, Japanese researchers isolated the antibiotic fosmidomycin (**1.83**, Figure 1.23) from *Streptomyces lavendulae*. It proved to be effective for the treatment of urogenital tract infections in an early phase II study (see 1.2.4.5.). With the emergence of more potent antibiotics, further development on fosmidomycin was discontinued. The mechanism of action was unknown until 1998 when fosmidomycin and its derivative FR900098 (**1.84**, Figure 1.23) were shown to represent highly specific inhibitors of DXR, the second enzyme of the DOXP pathway.<sup>169,170</sup>

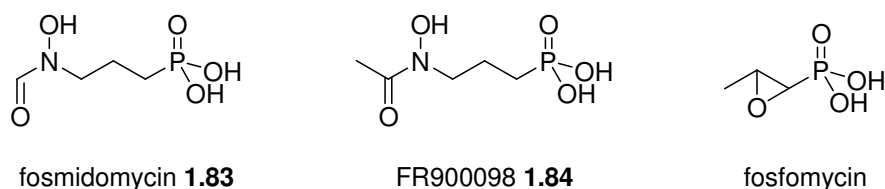


Figure 1.23: Structure of fosmidomycin, FR900098 and fosfomycin.

The presence of the DOXP and/or mevalonate pathway in some bacteria and the corresponding MIC values of fosmidomycin are summarized in table 1.1.

	DOXP pathway	DXR	Mevalonate pathway	MIC (50%)(μg/ml) fosmidomycin ( <b>1.83</b> )
<i>Escherichia coli</i>	present	present	absent	1.56
<i>Pseudomonas aeruginosa</i>	present	present	present	0.39-6.25*
<i>Salmonella typhi</i>	present	present	???	6.25
<i>Staphylococcus aureus</i>	certain enzymes	absent	present	>100
<i>Neisseria gonorrhoea</i>	present	present	???	>100
<i>Clostridium difficile</i>	present	present	certain enzymes	>100
<i>Mycobacterium tuberculosis</i>	present	present	absent	>100

Table 1.1: Presence or absence of the DOXP and/or mevalonate pathway in some bacteria and the corresponding MIC values of fosmidomycin (\* sensitivity for fosmidomycin is variable)<sup>171,172</sup>

<sup>169</sup> Zeidler, J., Schwender, J., Muller, C., Wiesner, J., Weidemeyer, C., Beck, E., Jomaa, H. Z. *Naturforsch., Teil C* **1998**, 53, 980-986.

<sup>170</sup> Kuzuyama, T., Shimizu, T., Takahashi, S., Seto, H. *Tetrahedron Lett.* **1998**, 39, 7913-7916.

From Table 1.1 it can be deduced that there is no absolute correlation between the presence of the DOXP pathway and the sensitivity to fosmidomycin. This conclusion can be drawn from two different studies.<sup>171,172</sup> The mechanism of non-sensitivity or resistance to fosmidomycin could be based on three principles.

- The absence of the target DXR.
- The absence of a glycerol 3-phosphate transporter (*glp T*) activity. Indeed, in *E. coli*, fosmidomycin is transported into the bacterium via this transporter. Mutations in the *glp T* gene are linked to resistance to fosmidomycin and fosfomycin (Figure 1.23). Fosfomycin is a well-known antibiotic which also contains a C–P bond and disturbs the formation of peptidoglycan and bacterial cell wall synthesis.<sup>173,174</sup> Based on their structural similarity, both antibiotics are brought in the bacterial cell by the same transport system. Therefore, simultaneous resistance of both phosphonate antibiotics is often seen.<sup>171</sup> The transcription of the *glpT* gene is highly dependent on the presence of cAMP. The gene is part of the *glpTQ*-operon which contains a cAMP receptor protein (CRP)-binding box. *E. coli* strains which are deficient in adenylate cyclase show fosmidomycin resistance, which can be reversed by adding cAMP to the medium.<sup>175</sup> Fosmidomycin-resistant strains of *P. aeruginosa* all proved deficient in *glp T* activity.<sup>176</sup> Recombinant DXR from *Mycobacterium tuberculosis* (*Rv2870c*) and recombinant DXR from *E. coli* (*lspC*) are very similar. The DXR of *M. tuberculosis* is only active in the presence of a divalent magnesium cation. Nevertheless *M. tuberculosis* is resistant to concentrations of fosmidomycin that are at least 3 orders of magnitude greater than the IC<sub>50</sub> determined *in vitro* for DXR. Indeed, analysis of the genome of *M. tuberculosis* demonstrates the absence of genes that encode for proteins with significant

<sup>171</sup> Neu, H. C., Kamimura, T. *Antimicrob. Agents Chemother.* **1981**, 19, 1013-1023.

<sup>172</sup> Boucher, Y., Doolittle, W. F. *Mol. Microbiol.* **2000**, 37, 703-716.

<sup>173</sup> Hendlin, D., Stapley, E. O., Jackson, M., Wallick, H., Miller, A. K., Wolf, F. J., Miller, T. W., Chaiet, L., Kahan, F. K., Foltz, E. L., Woodruff, H. B., Mata, J. M., Hernandez, S., Mochales, S. *Science* **1969**, 166, 122-123.

<sup>174</sup> Christensen, B. G., Leanza, W. J., Beattie, T. R., Patchett, A. A., Arison, B. H., Omrond, R. E., Kuehl, Jr. F. A., Albers-Schonberg, G., Jardetzky, O. *Science* **1969**, 166, 123-125.

<sup>175</sup> Sakamoto, Y., Furukawa, S., Ogihara, H., Yamasaki, M. *Biosci. Biotechnol. Biochem.* **2003**, 67, 2030-2033.

<sup>176</sup> Kojo, H., Shigi, Y., Nishida, M. *J. Antibiot.* **1980**, 33, 44-48.

similarity in primary structure to *glpT*.<sup>177</sup> Attempts to express *glpT* in *M. tuberculosis* have been unsuccessful. It is however possible that metabolic transformation or efflux mechanisms contribute to the observed resistance.

- As a result of efforts to find a gene related to the transport mechanism of fosmidomycin other than *glpT*, *fsr* was discovered. It encodes for an efflux protein with 409 amino acids and a molecular weight of 43303 Da. *Fsr* can cause resistance by transformation of sensitive strains. The transformation of *E. coli* DH5 with two different plasmids, pTH4ΔN (with a high-copy-number) and pIT1 (with a low-copy-number), increases the MIC from 6 µg/ml to 400 µg/ml and 25 µg/ml respectively. Although, the increase in the level of the *fsr* protein seems to have no direct effect on the levels of isoprenoids. The *fsr* protein contains 12 lipophilic transmembrane segments, which are also present in other antibiotic-export proteins.<sup>178</sup>

#### 1.2.4.2. SENSITIVITY OF *P. FALCIPARUM* STRAINS TOWARDS FOSMIDOMYCIN, FR900098 AND CHLOROQUINE

IC<sub>50</sub> values of fosmidomycin and FR900098 on different *P. falciparum* strains (HB3, Honduras, A2, Gambia, and Dd2, Indochina) are summarized in Table 1.2 and compared with chloroquine. FR900098 is more active than fosmidomycin, but 5 to 10 times less active than chloroquine against chloroquine sensitive strains. It is however twice as active as chloroquine against the chloroquine resistant Dd2 strain.

<i>P. falciparum</i> strain	IC <sub>50</sub> (nM)		
	fosmidomycin	FR900098	chloroquine
HB3	350 ± 170	170 ± 100	20 ± 5
A2	370 ± 45	171 ± 45	37 ± 7
Dd2	290 ± 130	90 ± 20	200 ± 30

Numbers are mean values ± standard deviations

Tabel 1.2: *In vitro* antimalarial activity of fosmidomycin and FR900098 against three different *P. falciparum* strains.<sup>1</sup>

<sup>177</sup> Dhiman, R. K., Schaeffer, M. L., Bailey, A. M., Testa, C. A., Scherman, H., Crick, D. C. *J. Bacteriol.* **2005**, *187*, 8395-8402.

<sup>178</sup> Fujisaki, S., Ohnuma, S., Horiuchi, T., Takahashi, I., Tsukui, S., Nishimura, Y., Nishino, T., Kitabatake, M., Inokuchi, H. *Gene* **1996**, *175*, 83-87.

### 1.2.4.3. PHARMACOKINETICS OF FOSMIDOMYCIN

In a murine malaria model, mice infected with *P. vinckei* were cured by fosmidomycin after oral (p.o.) or intraperitoneal (i.p.) administration. The efficacy was lower upon oral administration presumably due to a poor absorption rate.<sup>179</sup>

The plasma concentrations of fosmidomycin (Figure 1.23) after intravenous (i.v.) administration of 30 mg/kg ( $\pm$  2.2 g), intramuscular (i.m.) administration of 7.5 mg/kg ( $\pm$  560 mg) and p.o. administration of 500 mg fosmidomycin in healthy adult male volunteers is shown in Figure 1.24.

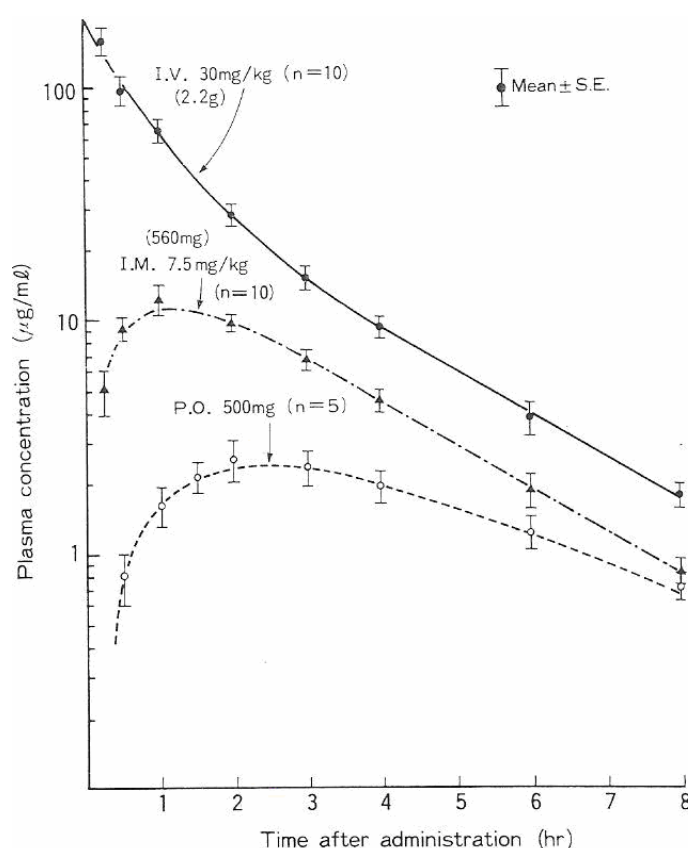


Figure 1.24: Plasma concentrations of fosmidomycin after a single dosis (from ref. 181).

The AUC's are respectively 210, 42.2 and 14.0  $\mu\text{g}\cdot\text{h}/\text{ml}$ . Phase I study results indicate that fosmidomycin is well tolerated in healthy adult male volunteers even when given in repeated doses of 8g/day i.v. for 7 days, 4 g/day i.m. for 5 days, and 4

<sup>179</sup> Kuemmerle, H. P., Murakawa, T., De Santis, F. *Chemioterapia* **1987**, 6, 113-119.



g/day p.o. for 7 days.<sup>180</sup> As shown in Figure 1.24 a peak serum concentration of 2.33 mg/l was reached at 2.36 h after oral administration. The plasma half-life was 1.87 h, which is very short and not very useful in a therapeutic setting. The biological availability is 80.3% after i.m. administration and 30% after oral administration. After oral administration of 500 mg fosmidomycin the gastrointestinal absorption rate was between 20-40%. The recovery rate in urine was 85.5%, 66.4% and 26% after i.v., i.m. and p.o. administration, respectively. There are no other active metabolites found in the urine. In repeated dose studies, no accumulation could be observed and the serum protein binding was found to be less than 1% in man.<sup>181,182</sup>

#### 1.2.4.4. TOXICOLOGY

The remarkably low toxicity is one of the major advantages of fosmidomycin. The LD<sub>50</sub> in rats is approximately 12 g/kg and 8g/kg after oral and subcutaneous administration, respectively.<sup>183</sup> In the phase I clinical trials, the effect of fosmidomycin was investigated after oral administration in single dose to 127 healthy male volunteers.<sup>180</sup> Administered doses were 250 mg to 1 g p.o., 1 g i.m. and several doses to 4 g i.v.. No changes in haematological (WBC, RBC, thrombocytes, hemoglobine, hematocrit) and biochemical (ureum, creatinine, total proteins, bilirubine, alkaline phosphatase, AST, ALT, glucose, iron, cholesterol, triglycerides, Ca<sup>2+</sup>, PO<sub>4</sub><sup>3-</sup>) parameters were observed. No adverse side-effects were observed in ECG, heartbeat, blood pressure and breath rythmn. Also no adverse events in urine investigation (pH, ketones, bilirubine, urobilinogene, glucose, proteins, blood) were reported. When administered in repeated doses of 8 g/day i.v. for 7 days, 4 g/day i.m. for 5 days and 4 g/day p.o. for 7 days, no adverse events were reported. Alterations in the intestinal flora caused mild side effects, such as loose stools, flatulence and diarrhoea.

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<sup>180</sup> Kuemmerle, H. P., Murakawa, T., Soneoka, K., Konishi, T. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **1985**, 23, 515-520.

<sup>181</sup> Kuemmerle, H. P., Murakawa, T., Soneoka, K., Konishi, T. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **1985**, 23, 521-528.

<sup>182</sup> Murakawa, T., Sakamoto, H., Fukada, S., Konishi, T., Nishida, M. *Antimicrob. Agents Chemother.* **1982**, 21, 224-230.

<sup>183</sup> Kamiya, T., Hashimoto, M., Hemmi, K., Takeno, H. US-patent no. 4206156.

#### 1.2.4.5. CLINICAL TRIALS

Fosmidomycin was already evaluated in the mid 1980s in an early phase I study and a phase II study for the management of urinary tract infections (UTI). The efficacy was good and no adverse side effects were observed.<sup>181</sup>

In recent clinical trials conducted in Gabon and Thailand fosmidomycin was examined as an antimalarial agent.<sup>184</sup> Then patients suffering from acute uncomplicated *P. falciparum* infection, were treated with 1200 mg of fosmidomycin three times a day for 7 days. The drug was very well tolerated and effective against multi-resistant *P. falciparum* parasite strains. Only mild side effects, such as diarrhoea, were reported, probably due to the antibacterial activity of fosmidomycin. The parasite and fever clearance times were approximately 2 days and similar to that of common antimalarials such as chloroquine or mefloquine. In another study, the duration of the treatment was shortened to 4 days and cure rates higher than 80% on day 14 were observed.<sup>185</sup> However, a high recrudescence rate was observed when fosmidomycin was given in monotherapy, possibly as a consequence of its extremely short plasma half-life. A complete cure could be achieved by combining fosmidomycin with artesunate or clindamycin. After trials with several currently used antimalarial drugs, clindamycin appeared a suitable combination partner.<sup>186</sup> Fosmidomycin has been found to act synergistically with clindamycin, as well as with artesunate. In many cases synergistic interactions are seen when multiple drugs target the same metabolic pathway, even when the actual enzyme targets are quite distant from each other. The activity of some bisphosphonates (farnesyl pyrophosphate synthase inhibitors) is also strongly potentiated by fosmidomycin.<sup>135(b)</sup>

Very recent studies in Gabon indicate that a three-day treatment of children (7-14 year) with fosmidomycin-clindamycin is superior to the standard therapy with chloroquine or sulfadoxine-pyrimethamin.<sup>187,188,189</sup> The synergistic effect of

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<sup>184</sup> Lell, B., Ruangweerayut, R., Wiesner, J., Missinou, M. A., Schindler, A., Baranek, T., Hintz, M., Hutchinson, D., Jomaa, H., Kremsner, P. G. *Antimicrob. Agents Chemother.* **2003**, *47*, 735-738.

<sup>185</sup> Missinou, M. A., Borrmann, S., Schindler, A., Issifou, S., Adegnika, A., Matsiegui, P. B., Binder, R., Lell, B., Wiesner, J., Baranek, T., Jomaa, H., Kremsner, P. G. *Lancet* **2002**, *360*, 1941-1942.

<sup>186</sup> Wiesner, J., Henschker, D., Hutchinson, D. B., Beck, E., Jomaa, H. *Antimicrob. Agents Chemother.* **2002**, *46*, 2889-2894.

<sup>187</sup> Borrmann, S., Adegnika, A. A., Matsiegui, P.-B., Issifou, S., Schindler, A., Mawili-Mboumba, D. P., Baranek, T., Wiesner, J., Jomaa, H., Kremsner, P. G. *J. Infect. Dis.* **2004**, *189*, 901-908.

clindamycin is probably due to its inhibition of the replication of the apicoplast, the organelle that harbors the enzymes of the DOXP pathway. A combination of fosmidomycin with artesunate (children 7-14 year) gave cure rates of 95% by day 14 and 100% by day 28, respectively with a 2 day and 3 day regimen.<sup>190</sup> Also in these clinical trials no adverse side-effects were reported.

### **1.2.5. 1-DEOXY-D-XYLULOSE-5-PHOSPHATE REDUCTOISOMERASE (DXR, ISPC)**

#### **1.2.5.1. MECHANISTIC STUDIES OF DXR**

DXR is responsible for the conversion of DOXP into MEP. The reaction of DXR consists of two steps (Figure 1.25). It is accepted that DOXP is first isomerized into 2-*C*-methyl-D-erythrose 4-phosphate, which is subsequently converted into MEP. In the first step, the C3–C4 bond in DOXP is cleaved with the direct formation of a bond between C2 and C4 to produce the 2-*C*-methyl-D-erythrose 4-phosphate intermediate, which is then reduced by NADPH into MEP. The aldehyde intermediate remains attached to the enzyme during the reaction, so it is impossible to detect it as a free molecule.<sup>166</sup>

Two possible mechanisms are suggested for the isomerisation: an  $\alpha$ -ketol rearrangement and a retroaldolization/aldolization. The  $\alpha$ -ketol rearrangement is similar to the mechanism proposed for ketol-acid reductoisomerase. Recently, Rohmer *et al.* suggested a retroaldolization/aldolization.<sup>191</sup> In the retroaldolization/aldolization, deprotonation of the C4-hydroxyl group, followed by cleavage of the C3–C4 bond gives glycoaldehyde phosphate and the enol(ate) of hydroxyacetone. Subsequent aldolization gives the intermediate. The most relevant

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<sup>188</sup> Borrmann, S., Issifou, S., Esser, G., Adegnika, A. A., Ramharter, M., Matsiegui, P.–B., Oyakhirome, S., Mawili-Mboumba, D. P., Missinou, M. A., Kun, J. F. J., Jomaa, H., Kremsner, P. G. *J. Infect. Dis.* **2004**, *190*, 1534-1540.

<sup>189</sup> Borrmann, S., Lundgren, I., Oyakhirome, S., Impouma, B., Matsiegui, P.–B., Adegnika, A. A., Issifou, S., Kun, J. F. J., Hutchinson, D., Wiesner, J., Jomaa, H., Kremsner, P. G. *Antimicrob. Agents Chemother.* **2006**, *50*, 2713-2718.

<sup>190</sup> Borrmann, S., Adegnika, A. A., Moussavou, F., Oyakhirome, S., Esser, G., Matsiegui, P.–B., Ramharter, M., Lundgren, I., Kombila, M., Issifou, S., Hutchinson, D., Wiesner, J., Jomaa, H., Kremsner, P. G. *Antimicrob. Agents Chemother.* **2005**, *49*, 3749-3754.

<sup>191</sup> Hoeffler, J. F., Tritsch, D., Grosdemange-Billiard, C., Rohmer, M. *Eur. J. Biochem.* **2002**, *269*, 4446-4457.

mechanism is still a matter of debate. As discussed in paragraph 1.3.1.4. several analogues were made to distinguish between both mechanisms.

The stereochemistry of the reduction step was studied by Proteau *et al.*<sup>192</sup> and Arigoni *et al.*,<sup>193</sup> who found that the *pro-S*-hydride from NADPH was delivered to the *re* face of the aldehyde. Based on the stereochemical features of the NADPH-dependent reduction, DXR is classified as a class B dehydrogenase.<sup>192,194</sup>

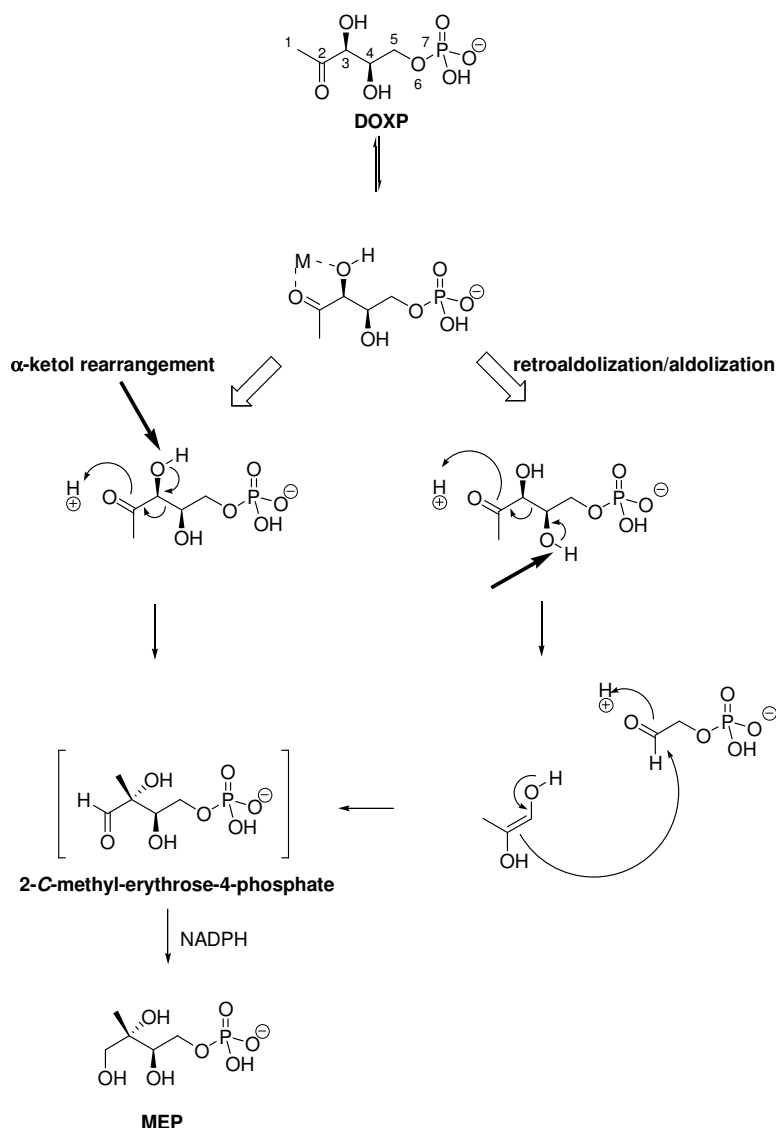


Figure 1.25: Two mechanisms proposed for DXR conversion of DOXP into 2-C-methyl-D-erythrose 4-phosphate.

<sup>192</sup> Proteau, P. J., Woo, Y.-H., Williamson, T., Phaosiri, C. *Org. Lett.* **1999**, 1, 921-923.

<sup>193</sup> Arigoni, D., Giner, J.-L., Sagner, S., Wungsintaweekul, J., Zenk, M. H., Kris, K., Bacher, A., Eisenreich, W. *Chem. Commun.* **1999**, 1127-1128.

<sup>194</sup> Radykewicz, T., Rohdich, F., Wungsintaweekul, J., Herz, S., Kis, K., Eisenreich, W. *FEBS Letters*, **2000**, 465, 157-160.

### 1.2.5.2. ENZYME KINETICS

Kinetic analysis of DXR shows that fosmidomycin binding is based on competitive/non-competitive inhibition mechanism. An analysis of the progress curves for MEP synthesis in the presence of inhibitor revealed a slow, tight-binding mode of inhibition by fosmidomycin and two kinetically distinguishable  $K_i$  values have been reported for the *E. coli* enzyme. A conformational change is induced after bonding of fosmidomycin to the enzyme (EI). This conformational change is essential for tight binding and requires prior formation of a DXR-NADPH complex.<sup>166</sup> The formation of additional hydrogen bonds between the enzyme and NADPH in the closed conformation correlates with observation that NADPH is essential for the strong binding to the inhibitor. Based on the slow timescale of DXR domain movement compared to the rate of inhibitor diffusion, it is possible to explain the slow onset of inhibition that has been observed for fosmidomycin.

### 1.2.5.3. 3D STRUCTURE OF DXR

Until now, four high and medium resolution X-ray structures of DXR from *E. coli* have been published. The first structure reported concerns the apo-enzyme (Figure 1.26).<sup>195</sup>

The second holo structure is a binary complex with NADPH and a sulphate ion in the active site.<sup>196</sup> These two reports are less useful for inhibitor design as they do not reveal any information on the interactions between ligand and enzyme.

DXR is a homodimer. Each monomer shows a V-like shape or saddle form and is composed of 3 domains. The amino-terminal dinucleotide binding domain (amino acids 1-150), which forms 1 arm of the V-like shape, consists of a  $\beta$  sheet with 7 parallel strands ( $\beta 1$ - $\beta 7$ ) which are connected by six  $\alpha$  helices ( $\alpha 1$ - $\alpha 6$ ). These are responsible for the binding of NADPH. The other arm of the saddle (amino acids 312-398) contains four  $\alpha$  helices ( $\alpha 12$ - $\alpha 15$ ), which form the carboxyl-terminal domain.

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<sup>195</sup> Reuter, K., Sanderbrand, S., Jomaa, H., Wiesner, J., Steinbrecher I., Beck, E., Hintz, M., Klebe, G., Stubbs, M. T. *J. Biol. Chem.* **2002**, 277, 5378-5384.

<sup>196</sup> Yajima, S., Nonaka, T., Kuzuyama, T., Seto, H., Ohsawa, K. *J. Biochem. (Tokyo)* **2002**, 131, 313-317.

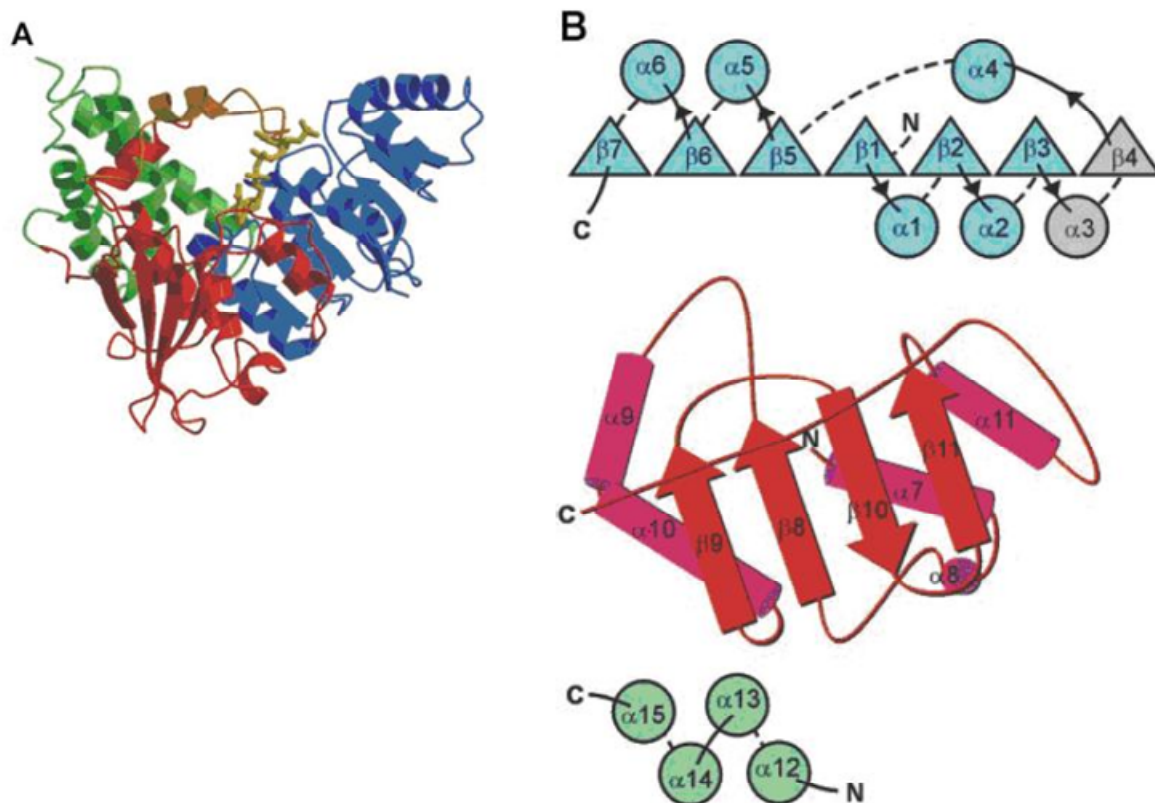


Figure 1.26: **(A)** X-ray structures of *E. coli* DXR with NADPH modelled into the structure. Colored in blue: the dinucleotide binding domain, colored in green: the carboxyl-terminal four-helix bundle domain; colored in red: the connective domain. Colored in ochre: the flexible loop. **(B)** Topology diagram of DXR. The color code is the same as in **(A)**. (Figure from ref. 195)

These 2 domains are connected by a four-stranded  $\beta$  sheet ( $\beta 8$ - $\beta 11$ ), in turn connected by five  $\alpha$  helices ( $\alpha 7$ - $\alpha 11$ ) (Figure 1.26). The molecule can move around this domain, which can be considered as a kind of hinge. This part also comprises the active site of the enzyme. It contains the binding site for the divalent cation, for the phosphate part of the substrate and also a flexible loop (amino acids 186-216). There are two essential conformations. In one conformation the loop hangs over the active site and touches the dinucleotide binding domain. In the other conformation the flexible loop hardly bulges out of the active site and folds back towards the four helix bundle. This last conformation is probably the substrate binding conformation and the first conformation is the active conformation where the flexible loop acts as a kind of lid. Plausibly, an interaction between the dinucleotide and His257 and Trp212

is important. The two monomers form a dimer and are connected via  $\beta$ 11 (in both monomers) and the loop, which connects  $\alpha$ -helix 11 and  $\alpha$ -helix 12, i.e. the apical part of the V. The substrate is believed to bind in the cavity between the dinucleotide binding domain and the four carboxyl terminal helices.

Next, Steinbacher *et al.* reported a complex DXR with fosmidomycin and a  $Mn^{2+}$  ion, but without the cofactor.<sup>197</sup> In Steinbacher's model (Figure 1.27)  $Mn^{2+}$  is bound to a cluster of the carboxylic acids Asp 150, Glu 152 and Glu231. All residues form monodentate ligands and a regular octahedral coordination sphere is completed by water molecules. The *N*-formyl oxygen of fosmidomycin is found in the trans position to Glu231, whereas the *N*-hydroxyl oxygen is located trans to Asp150. The phosphonate moiety is anchored by numerous hydrogen bonds to Ser186, Ser222, Asn227 and Lys228. According to this study hydrophobic contacts of the linker do not contribute to the high affinity of the inhibitor. Steinbacher's model represents the structure of fosmidomycin bound to DXR in its open conformation.

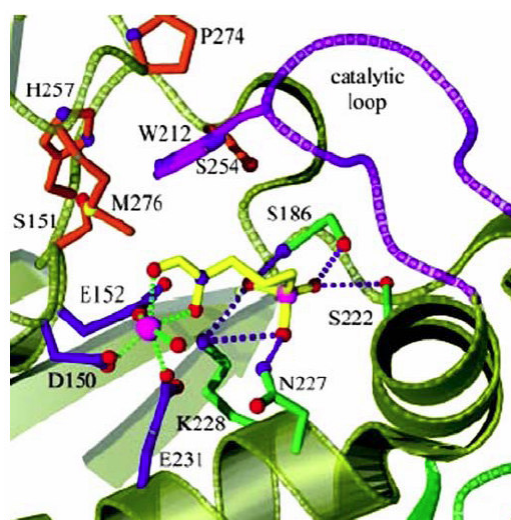


Figure 1.27: Binding of fosmidomycin on the active site of DXR. Fosmidomycin is colored in yellow, the catalytic loop of DXR in purple, the divalent cation in pink and important amino acids in the active site are colored in blue, green and orange. The *N*-formyl oxygen is located trans of Glu231 and the *N*-hydroxy oxygen is located trans of Asp-150. The phosphonate group forms hydrogen bonding to Ser186, Ser222, Asn227 and Lys228. (Figure from ref.

197)

<sup>197</sup> Steinbacher, S., Kaiser, J., Eisenreich, W., Huber, R., Bacher, A., Rohdich, F. *J. Biol. Chem.* **2003**, 278, 18401-18407.

Most recently, Mac Sweeney *et al.* reported the ternary structures of a wild-type and SeMet-labelled DXR, cocrystallized with fosmidomycin, magnesium and the cofactor NADPH. This reveals a binding mode that is not mediated by a metal ion. The absence of the expected metal ion from the structure can be explained by the low metal affinity of DXR in the crystallization buffer (pH = 5). The ternary complex of the DXR, NADPH and the substrate DOXP is also presented and was used as model of metal-mediated fosmidomycin binding.<sup>198</sup> By contrast to the open conformation of Steinbacher's model, Mac Sweeney *et al.* proposed a tight-binding conformation where fosmidomycin is buried in a solvent-shielded binding site. Hydrophobic interactions were observed with the highly conserved residues Trp212, Met214 and Met276 (Figure 1.28 **A**). The nicotinamide ring of NADPH also contributes to the formation of a hydrophobic binding pocket.

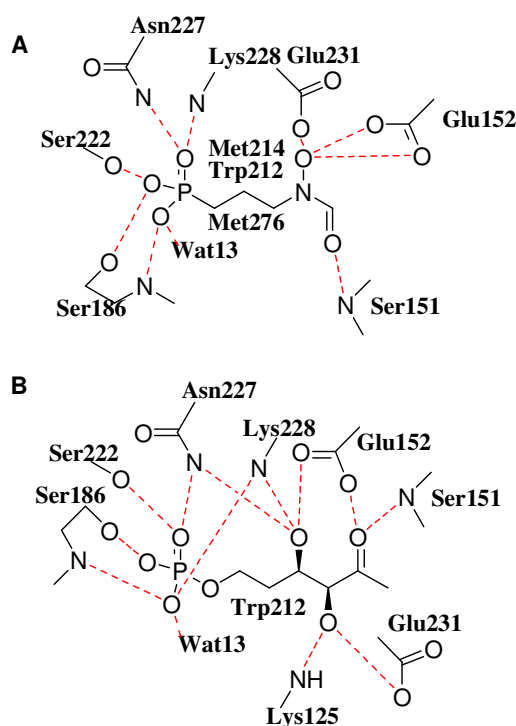


Figure 1.28: **(A)** Simplified schematic diagram of fosmidomycin binding to DXR. **(B)** Simplified schematic diagram of DOXP binding to DXR.

The DOXP backbone (Figure 1.28 **B**) interacts with the  $\beta$ -indole of Trp212 and the carbonyl group of C2 is hydrogen bonded to Glu 152 and the nitrogen of Ser151.

<sup>198</sup> Mac Sweeney, A., Lange, R., Fernandes, R. P. M., Schulz, H., Dale, G. E., Douangamath, A., Proteau, P. J., Oefner, C. J. *Mol. Biol.* **2005**, 345, 115-127.



The hydroxyl group of C3 is involved in hydrogen bonds with Lys125 and Glu231, while the C4 hydroxyl group is hydrogen bonded to Glu152, Asn227 and Lys228.

The most remarkable difference between the ternary complexes in the presence of NADPH and the other structures of DXR is that in the former an induced fit adaptation of the connective and C-terminal domains upon small molecule binding was observed. This resulted in a closed enzyme conformation, which is in agreement with the 2-step binding mode of fosmidomycin as proposed by Koppisch and coworkers.<sup>166</sup> In addition, the flexible loop connecting both domains (residues 206-216) undergoes an induced fit adaptation and adopts a conformation that allows it to function as a lid over the active site. As a result, the active site is shielded from the solvent. The  $\beta$ -indole of Trp212 provides the key interaction with the substrate or fosmidomycin backbone.<sup>198</sup> The fact that the same enzyme conformation was observed in both ternary complexes, despite the different crystallization conditions, indicates that the closed conformation represents most likely the physiologically relevant complex in solution. The open conformation in complex with fosmidomycin represents the initial, lower affinity enzyme-inhibitor complex. The ternary complexes also reveal a difference in binding mode between inhibitor and substrate. Also certain amino acids in the binding site, such as His209 could play a role in the “pre-orientation” of the substrate in a conformation that favors retroaldolization/aldolization or  $\alpha$ -ketol rearrangement.<sup>198</sup>

Based on these data, it may be concluded that the enzyme can adopt several conformations. Although the Mac Sweeney structure provides important information, a main drawback is that the enzyme is not catalytically active at the crystallization pH of 5 and that the protein-ligand interactions may differ from the *in vivo* situation. Therefore, conclusions with respect to the putative protein-ligand interactions should be drawn very carefully. Recently, Silber *et al.* reported a computer based AFMoC study as an *in silico* predictor of the binding affinity of ligands to DXR.<sup>199(a)</sup> A computational approach to determine the 3D structure of *P.falciparum* DXR was also described. Docking studies were carried out on known DXR inhibitors using the modeled *P.falciparum* DXR, and a correlation was obtained between ligand binding affinity and empirical docking score.<sup>199(b)</sup>

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<sup>199</sup> (a) Silber, K., Heidler, P., Kurz, T., Klebe, G. *J. Med. Chem.* **2005**, 48, 3547-3563. (b) Singh, N., Chevé, G., Avery, M. A., McCurdy, C. R. *J. Chem. Inf. Model.* **2006**, 46, 1360-1370.

Furthermore, there is also a complex reported with two bisphosphonate inhibitors and manganese bound to DXR. These inhibitors appear to bind in a completely different fashion than fosmidomycin.<sup>200</sup> The DXR structure of *Zymomonas mobilis* is also solved. Unfortunately only the apo structure and the DXR-NADPH complex are reported.<sup>201</sup> The active site of the DXR of both organisms shows high similarity.

#### 1.2.5.4. MODE OF ACTION OF FOSMIDOMYCIN

Fosmidomycin acts as a transition state analogue with the retrohydroxamic acid moiety chelating the metal ion and the phosphonate group resembling the phosphate group of the substrate (Figure 1.29). The X-ray structure of DXR complexed with fosmidomycin and a divalent cation,<sup>197</sup> reveals that the *N*-formyl oxygen and the *N*-hydroxy oxygen of fosmidomycin displace two water molecules that were bound to the divalent cation (Figure 1.27). The divalent cation is required for the reduction step but whether it is required for the initial isomerisation step has not been elucidated yet.

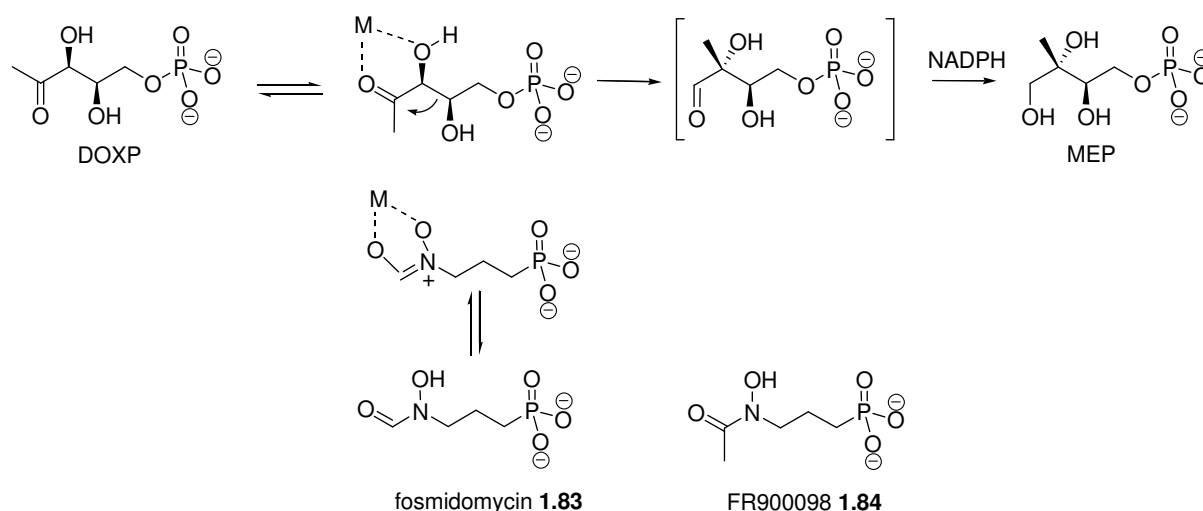


Figure 1.29: Mechanism of the conversion of DOXP into MEP and the complexation of the divalent cation by fosmidomycin (**1.83**). FR900098 (**1.84**) acts in the same way.

<sup>200</sup> Yajima, S., Hara, K., Sanders, J. M., Yin, F., Ohsawa, K., Wiesner, J., Jomaa, H., Oldfield, E. *J. Am. Chem. Soc.* **2004**, *126*, 10824-10825.

<sup>201</sup> Ricagno, S., Grolle, S., Bringer-Meyer, S., Sahm, H., Lindqvist, Y., *et al. Biochim. Biophys. Acta* **2004**, *1698*, 37-44.

Replacement of the formyl-hydrogen of fosmidomycin by a methyl group yields FR900098 (**1.84**). The methyl group of FR900098 is predicted to contact the side chain of Trp212 of the catalytic loop (Figure 1.28). The corresponding methyl group is also present in DOXP, which supports the view that fosmidomycin binds substrate-like and not intermediate-like (Figure 1.30).<sup>197</sup> This is also supported by the higher affinity of FR900098 compared to fosmidomycin.<sup>1</sup> Indeed, the extra methyl group can be superimposed upon DOXP (Figure 1.29).

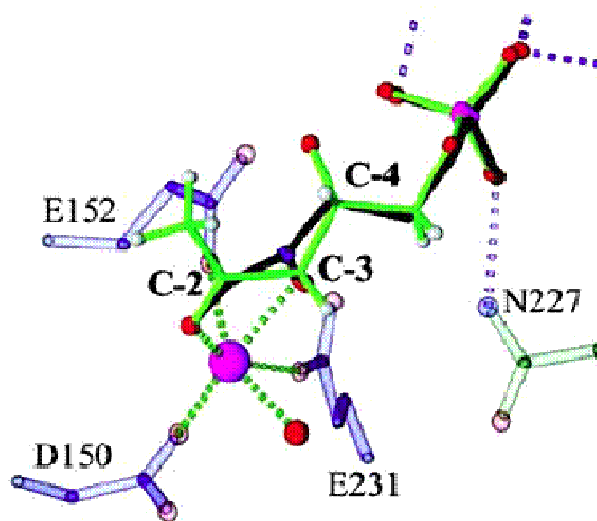


Figure 1.30: Comparison between bonding of DOXP (green) and fosmidomycin (black) with DXR. (Figure from ref. 197)

## 1.3. FOSMIDOMYCIN AS A LEAD IN DRUG DESIGN

### 1.3.1. ANALOGUES OF FOSMIDOMYCIN

The relatively high doses of fosmidomycin required for parasite clearance compared with chloroquine and the unfavourable pharmacokinetic properties prompted researchers to modify this lead. Fosmidomycin consists of a phosphonate and a retrohydroxamate moiety connected by a three carbon spacer and structural modifications at these positions have been considered. In the next paragraphs, we give an overview of the reported modifications.

### 1.3.1.1. MODIFICATIONS OF THE RETROHYDROXAMATE MOIETY

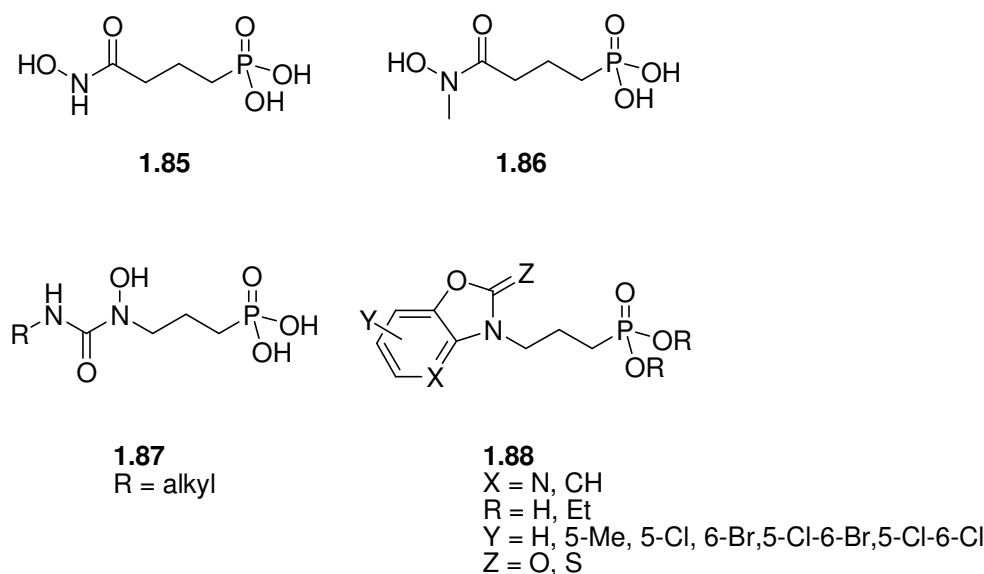


Figure 1.31: Structures of fosmidomycin analogues with an altered retrohydroxamate moiety.

Hydroxamic acids are known to have strong metal ion chelating properties.<sup>202</sup> The activity of several matrix metalloproteinase-, cyclooxygenase- and lipoxygenase inhibitors relies on the presence of an hydroxamic moiety.<sup>203</sup> The same is true for several antibacterial and antifungal agents.<sup>204</sup> Hydroxamic acids are also responsible for the activity of several inhibitors of angiotensin converting enzyme, neutral endopeptidase, urease and peptide deformylase.<sup>205</sup>

Hydroxamic acids **1.85** and **1.86** were synthesized and compared to fosmidomycin. Analogue **1.86** showed a high inhibitory activity towards *E. coli* DXR ( $K_i = 54$  nM vs 40 nM for fosmidomycin).<sup>206</sup>

<sup>202</sup> (a) Kurzak, B., Kozłowski, H., Farkas, E. *Coord. Chem. Rev.* **1992**, 114, 169-200. (b) Boukhris, S., Souizi, A., Robert, A. *Tetrahedron Lett.* **1996**, 37, 179-182.

<sup>203</sup> (a) Whittaker, M., Floyd, C. D., Brown, P., Gearing, A. J. H. *Chem. Rev.* **1999**, 99, 2735-2776. (b) Cheng, M., De, B., Pikul, S., Almstead, N. G., Natchus, M. G., Anastasio, M. V., McPhail, S. J., Snider, C. E., Taiwo, Y. O., Chen, L., Dunaway, C. M., Gu, F., Dowty, M. E., Mieling, G. E., Janusz, M. J., Wang-Weigand, S. *J. Med. Chem.* **2000**, 43, 369-380 and references therein.

<sup>204</sup> (a) Weber, G. *Cancer Res.* **1983**, 43, 3466-3492. (b) Miller, M. J. *Acc. Chem. Res.* **1986**, 19, 49-56. (c) Miller, M. J. *Chem. Rev.* **1989**, 89, 1563-1579.

<sup>205</sup> Marmion, C. J., Griffith, D., Nolan, K. B. *Eur. J. Inorg. Chem.* **2004**, 3003-3016.

<sup>206</sup> Kuntz, L., Tritsch, D., Grosdemange-Billiard, C., Hemmerlin, A., Willem, A., Bach, T.J., Rohmer, M. *Biochem. J.* **2005**, 386, 127-135.

In analogue **1.87** the retrohydroxamate group was replaced by a hydroxyurea moiety. Enzyme assays revealed that these analogues have no relevant inhibitory properties.<sup>207,208</sup>

Benzoxazolone or oxazolopyridinone analogues **1.88** were synthesized and tested as inhibitors for the ajmalicine production in plant cells of *Catharantus roseus*.<sup>209</sup> Ajmalicine represents a monoterpenoid indole alkaloid. Interestingly, four phosphonate diethyl esters ((X = CH, Y = 5-Cl, Z = O), (X = CH, Y = 5-Cl and 6-Br, Z = O), (X = CH, Y = 6-Cl, Z = S) and (X = N, Y = 6-Br, Z = O)) exerted a significant inhibition of ajmalicine accumulation at 125  $\mu$ M (45-85%).

### 1.3.1.2. MODIFICATIONS OF THE PROPYL CHAIN

The changes in the propyl chain are reported by the research laboratories of Fujisawa and date from the early 1980s (Figure 1.32). The MIC of analogues **1.89-1.94** was determined against *S. aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *E. coli* and *P. aeruginosa*.

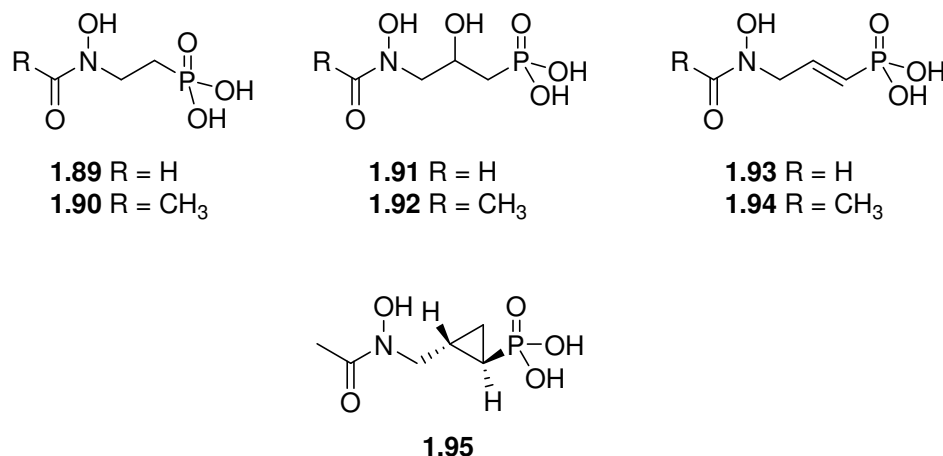


Figure 1.32: Overview of reported modifications of the propyl chain.

<sup>207</sup> Wienser, J., Jomaa, H. *unpublished results*.

<sup>208</sup> Kurz, T., Geffken, D., Wackendorf, C. *Z. Naturforsch. B* **2003**, 58, 106.

<sup>209</sup> (a) Mincheva, Z., Courtois, M., Andreu, F., Rideau, M., Viaud-Massuard, M.-C. *Phytochemistry* **2005**, 66, 1797-1803. (b) Courtois, M., Mincheva, Z., Andreu, F., Rideau, M., Viaud-Massuard, M.-C. *J. Enzym. Inhib. Med. Ch.* **2004**, 19, 559-565.

Shortening the length of the propylene spacer, such as in compounds **1.89** and **1.90**, compromised the antibacterial activity.<sup>210</sup> Likewise, no inhibitors of DXR that are significantly larger than fosmidomycin have been reported to date. This is consistent with the recently obtained X-ray structures.

The further screening of other congeners, isolated from *Streptomyces rubellomurius*, yielded 3-(*N*-acetyl-*N*-hydroxyamino)-2(*R*)-hydroxypropylphosphonic acid (**1.92**).<sup>211,212</sup> Analogues **1.91** and **1.92** showed no significant activity compared with fosmidomycin.

The Fujisawa laboratories also reported the synthesis of *N*-acyl derivatives of 3-(*N*-hydroxyamino)-1-*trans*-propenylphosphonic acid. Analogue **1.93** as well as fosmidomycin have been isolated from *Streptomyces lavendulae*. Analogue **1.94** showed a MIC against *E.coli* of 10 µg/ml compared with 12.5 µg/ml for fosmidomycin.<sup>212</sup>

Very recently, our group reported the synthesis and biological evaluation of a series of cyclopropane derivatives.<sup>213</sup> The cyclopropane derivative **1.95** with reduced conformational flexibility in the backbone was as active as fosmidomycin (**1.83**) (IC<sub>50</sub> = 480 nM on *P. falciparum* Dd2 strain) and slightly less active than FR900098 (**1.84**).

#### 1.3.1.3. MODIFICATIONS OF THE PHOSPHONATE MOIETY

Only a few modifications of the phosphonate functionality were reported (Figure 1.33). Kurz *et al.*<sup>214</sup> and Woo *et al.*<sup>215</sup> reported the synthesis of the carboxylic acid analogue **1.96**. The latter study comprised another analogue (**1.97**) with a sulfamate group. None of these analogues showed activity against DXR. These results suggest that the presence of two ionisable groups on the phosphonate group plays an important role in the biological activity.

<sup>210</sup> Hemmi, K., Takeno, H., Hashimoto, M., Kamiya, T. *Chem. Pharm. Bull.* **1982**, *30*, 111-118.

<sup>211</sup> Hemmi, K., Takeno, H., Hashimoto, M., Kamiya, T. *Chem. Pharm. Bull.* **1981**, *29*, 646-650.

<sup>212</sup> Hashimoto, M., Hemmi, K., Takeno, H., Kamiya, T. *Tetrahedron Lett.* **1980**, *21*, 99-102.

<sup>213</sup> Devreux, V., Wiesner, J., Goeman, J.L., Van der Eycken, J., Jomaa, H., Van Calenbergh, S. *J. Med. Chem.* **2006**, *49*, 2656-2660.

<sup>214</sup> Kurz, T., Geffken, D., Wackendorff, C. *Z. Naturforsch. B* **2003**, *58*, 457.

<sup>215</sup> Woo, Y.-H., Fernandes, R. P. M., Proteau, P. J. *Bioorg. Med. Chem.* **2005**, *14*, 2375-2385.

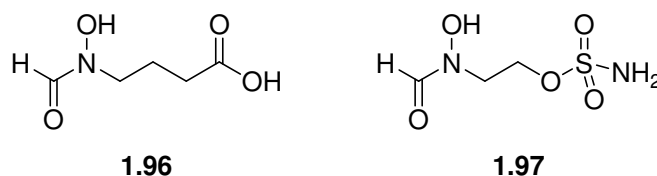


Figure 1.33: Analogues of fosmidomycin with a modified phosphonate moiety.

#### 1.3.1.4. SUBSTRATE OR TRANSITION STATE ANALOGUES

DXR binds NADPH and a divalent cation, which causes a slight conformational change, followed by binding of the substrate to cause a more drastic conformational change prior to the enzyme reaction. As discussed in paragraph 1.2.5.1. two different mechanisms are proposed for the rearrangement step, the formation of the 2-*C*-methyl-D-erythrose 4-phosphate intermediate. Several analogues of DOXP have been synthesized (Figure 1.34). Analogues that are accepted as substrate in the DXR-reaction could provide valuable information about the mechanism and the sterical requirements of the enzyme.

To distinguish between an  $\alpha$ -ketol and a retroaldolization mechanism, Hoeffler *et al.* have tested 3-deoxy and 4-deoxy analogues of DOXP as substrates for *E. coli* DXR.<sup>191</sup> An  $\alpha$ -ketol rearrangement is possible with the 4-deoxy analogue **1.98**. It was however not converted to the corresponding product, 3-deoxy methylerythritol phosphate. These findings point in the direction of a retroaldolization. Likewise, the 3-deoxy analogue of DOXP (**1.99**) was not accepted as a substrate. Instead, both deoxy-analogues behaved as mixed competitive/non-competitive inhibitors of DXR.

The same results were obtained upon replacement of the 3- or 4-position by a fluor atom (Figure 1.34).<sup>216</sup> The 4-fluoro-DOXP analogue (**1.100**) can theoretically undergo an  $\alpha$ -ketol rearrangement, but this reaction was not observed.

The 1-fluoro analogue of DOXP (**1.102**) appeared to be a good substrate ( $K_m$  = 227  $\mu$ M vs. DOXP = 50  $\mu$ M).<sup>216,217</sup> This pleads in favour of the retroaldolization, since the fluor could stabilize the negative charge arising in case of reaction in  $\beta$ -position of the fluor atom. Besides, in the case of an  $\alpha$ -ketol mechanism, the strongly

<sup>216</sup> Wong, A., Munos, J. W., Devasthali, V., Johnson, K. A., Liu, H. *Org. Lett.* **2004**, 6, 3625-3628.

<sup>217</sup> Fox, D. T., Poutler, C. D. *J. Org. Chem.* **2005**, 70, 1978-1985.

electron-withdrawing fluor atom would destabilize the positive charge on the aldehyde oxygen.

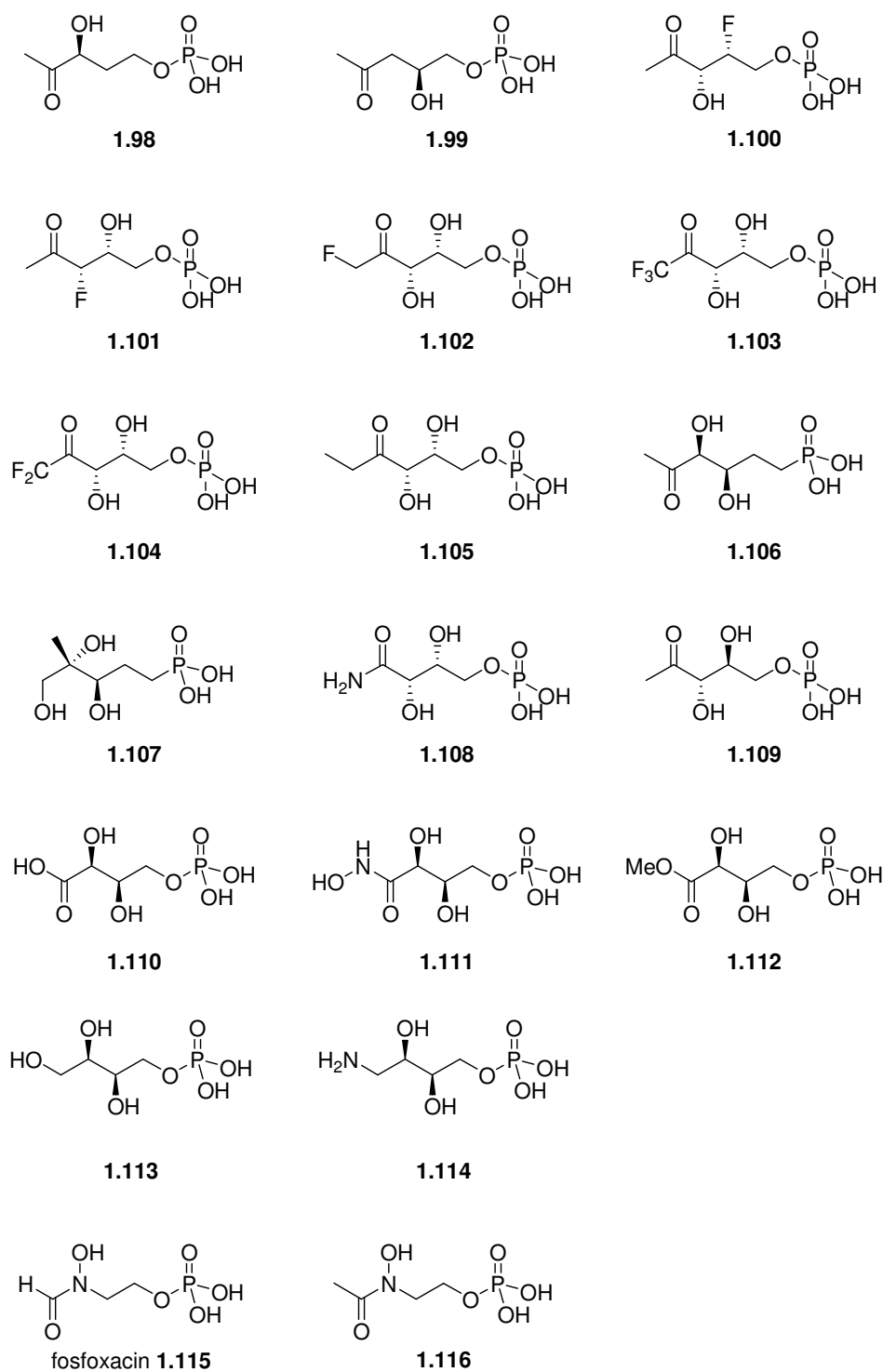


Figure 1.34: Overview of reported analogues of DOXP, MEP and the 2-*C*-methyl-D-erythrose 4-phosphate intermediate.



The other analogues, CF<sub>3</sub>-DOXP (**1.103**), CF<sub>2</sub>-DOXP (**1.104**) and Et-DOXP (**1.105**), reported by Fox *et al.*, were weak inhibitors of DXR. The presence of a hydrate form, which may not bind as tightly as the ketone, and their increase in steric bulk are possible reasons for this weak inhibition. These results illustrate that strict size requirements are present for the C1 position.

The phosphonate analogue of DOXP (**1.106**) did not show significant inhibitory activity of DXR. However, it was transformed into **1.107**, which is MEP with a C–P bond. Both compounds were synthesized by Rohmer and coworkers.<sup>218,219</sup>

The Et-DOXP (**1.105**), DOXP-phosphonate (**1.106**), 3-deoxy-DOXP (**1.99**) and 4-deoxy-DOXP (**1.98**) analogues were also reported by Phaosiri *et al.*,<sup>220</sup> together with two other analogues which were tested on *Synechocystis* PCC6803 DXR. One compound **1.108**, in which the methyl ketone moiety was replaced by an isosteric amido group, acted as a weak inhibitor because of the poorer electrophilicity at the carbonyl moiety. The  $K_i$  is about half of the  $K_m$  of DOXP, suggesting a more favourable binding, possibly through hydrogen bonding. Compound **1.109** with opposite stereochemistry at the C4 position relative to DOXP, had a  $K_i$  value comparable to the  $K_m$  of DOXP, suggesting that the stereochemistry at C4 of the substrate is not critical for binding, but is essential for catalysis.

In the assay with *Synechocystis* PCC6803 DXR, 3-deoxy-DOXP and 4-deoxy-DOXP behaved as competitive inhibitors, while Hoeffler *et al.* classified these deoxygenated compounds as mixed type inhibitors of *E. coli* DXR. The  $K_i$  of the deoxy analogues compared favourably to the  $K_m$  of DOXP demonstrating that the C3 and C4 OH are not critical for binding, but essential for catalysis.

The Et-DOXP binds *Synechocystis* PCC6803 DXR at the same site of DOXP, but apparently the added steric bulk prevents successful turnover. DOXP-phosphonate appears as an alternate substrate. The change from a phosphate to a phosphonate led to a drop in affinity but still allowed turnover probably because this modification is distant from the part of the molecule undergoing transformation.

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<sup>218</sup> Meyer, O., Grosdemange-Billiard, C., Tritsch, D., Rohmer, M. *Org. Biomol. Chem.* **2003**, 1, 4367-4372.

<sup>219</sup> Hirsch, G., Grosdemange-Billiard, C., Tritsch, D., Rohmer, M. *Tetrahedron Lett.* **2004**, 45, 519-521.

<sup>220</sup> Phaosiri, C., Proteau, P. J. *Bioorg. Med. Chem. Lett.* **2004**, 21, 5309-5312.

Recently, the amide **1.108** and five analogues (**1.110-1.114**) were prepared by Walker and Poulter.<sup>221</sup> In analogues **1.110-1.112** the methyl group of DOXP was replaced by hydroxyl, hydroxylamino and methoxy moieties, respectively. In analogues **1.113** and **1.114**, the acetyl group of DOXP was replaced by a hydroxymethyl and aminomethyl group. Except from analogues **1.111** and **1.114**, all were very weak inhibitors of *E. coli* DXR with IC<sub>50</sub> values from 0.25 to 1.0 mM. Analogue **1.111** was argued to be one atom too long to fit in the restricted volume of the pocket. In addition, Et-DOXP was confirmed to be a weak inhibitor. In derivative **1.114**, the amino group is supposed to be protonated and not able to complex the metal ion.

Recently, Proteau and coworkers described the synthesis of the phosphate analogues of fosmidomycin (**1.115**) and FR900098 (**1.116**).<sup>215</sup> The former had previously been isolated from *Pseudomonas fluorescens* PK-52 and is known as fosfoxacin.<sup>222</sup> The latter proved a very potent inhibitor of *Synechocystis* DXR ( $K_i = 0.002 \mu\text{M}$  vs. fosmidomycin =  $0.057 \mu\text{M}$ ) .

### 1.3.2. PRODRUGS

As a consequence of the strong hydrophilic properties, fosmidomycin shows a moderate biological availability of approximately 30%. At physiological pH, phosphonic acids occur in their ionised form and, therefore, pass poorly through membranes by passive diffusion. To enhance the *in vivo* availability of drugs with a phosphonic acid group, this group is often masked by a protective group. In this way a neutral and essentially lipophilic molecule is obtained. A requirement for the preservation of the activity is that the protective group is cleaved under physiological conditions to liberate the active form. Different prodrug derivatives of FR900098 (**1.84**) have been synthesized and evaluated.<sup>223,224</sup>

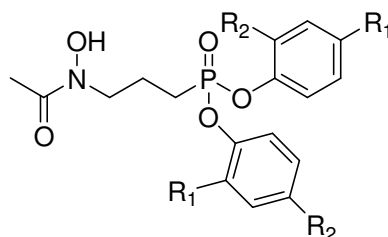
<sup>221</sup> Walker, J. R., Poulter, C. D. *J. Org. Chem.* **2005**, *70*, 9955-9959.

<sup>222</sup> Katayama, N., Tsubotani, S., Nozaki, Y., Harada, S., Ono, H. *J. Antibiot.* **1990**, *43*, 238-246.

<sup>223</sup> Noren, J. O., Helgstrand, E., Johansson, N. G., Misiorny, A., Stening, G. *J. Med. Chem.* **1983**, *26*, 264-270.

<sup>224</sup> Serafinowska, H. T., Ashton, R. J., Bailey, S., Harnden, M. R., Jackson, S. M., Sutton, D. *J. Med. Chem.* **1995**, *38*, 1372-1379.

### 1.3.2.1. DIARYL ESTERS



- 1.117**  $R_1 = H, R_2 = H$   
**1.118**  $R_1 = CH_3, R_2 = H$   
**1.119**  $R_1 = H, R_2 = OCH_3$

Figure 1.35: Diaryl ester prodrugs of FR900098.

The implementation of aryl and acyloxyalkyl esters in acyclic phosphononucleotides improved the bioavailability drastically.<sup>224</sup> Based on these results Reichenberg and coworkers transformed the phosphonate moiety of FR900098 into phosphodiaryl esters, expected to be hydrolysed by non-specific plasma esterases.<sup>225</sup> The antimalarial activity of the prodrugs (Figure 1.35) was tested in *Plasmodium vinckei*-infected mice.

The unsubstituted diphenyl ester **1.117** was significantly more active than FR900098 after oral administration. In contrast the bis-(2-methylphenyl) ester **1.118** was significantly less active than FR900098. Steric hindrance by the ortho substituent probably hampers access by the esterases. The bis-(4-methoxyphenyl) ester **1.119** proved the most efficient and equaled the activity of FR900098 administered by i.p. route.

### 1.3.2.2. ACYLOXYALKYL ESTERS

Concerns about the toxicity of the phenol derivatives generated by hydrolysis of the phenolic prodrugs prompted Ortmann and coworkers to make a series of

<sup>225</sup> Reichenberg, A., Wiesner, J., Weidemeyer, C., Dreiseidler, E., Sanderbrand, S., Altincicek, B., Beck, E., Schlitzer, M., Jomaa, H. *Bioorg. Med. Chem. Lett.* **2001**, 11, 833-835.

acyloxyalkyl ester prodrugs of FR900098 (Figure 1.36).<sup>226,227</sup> The activity of these prodrugs was tested in a same way as the diaryl esters. Prodrug **1.120** appeared less active and prodrug **1.121** was more active than FR900098. To avoid the formation of formaldehyde during hydrolysis, other analogues were prepared. The pivaloyloxyethyl ester **1.122** and the benzoyloxyethyl ester **1.123** displayed activities comparable to FR900098. The activities appeared to improve when less bulky acyl residues were used. Both the acetyloxyethyl ester **1.124** and the propionyloxyethyl ester **1.125** proved more effective than FR900098. In contrast, the propionyloxyisobutyl derivative **1.126** caused an activity drop. Treatment with 20 mg/kg **1.124** p.o., was slightly more effective than 40 mg/kg p.o. FR900098. This difference was due to higher plasma concentrations of FR900098. The two bis[(alkoxycarbonyloxy)ethyl] esters **1.127** and **1.128** were more active than FR900098. Until today, all these prodrugs were only tested in murine models. Compound **1.129** showed lower activity.

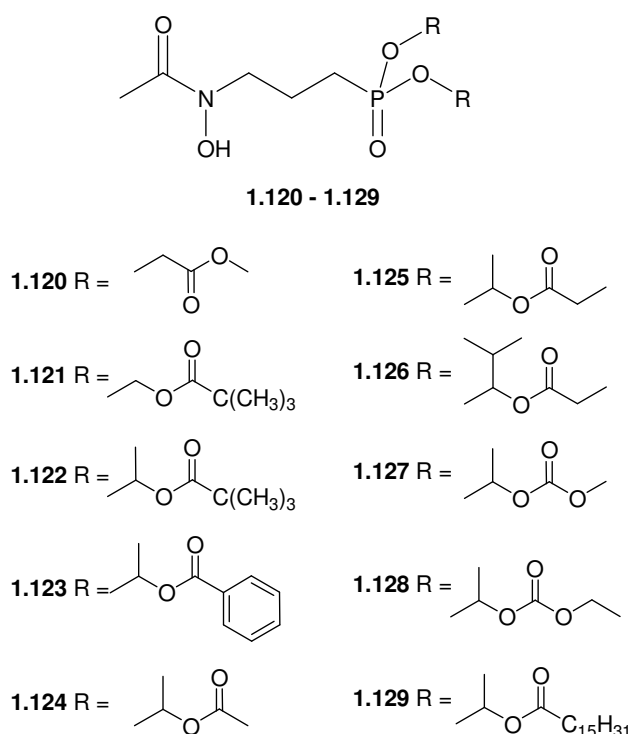


Figure 1.36: Overview of reported alkoxy carbonyloxyethyl ester prodrugs of FR900098.

<sup>226</sup> Ortmann, R., Wiesner, J., Reichenberg, A., Henschker, D., Beck, E., Jomaa, H., Schlitzer, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2163-2166.

<sup>227</sup> Ortmann, R., Wiesner, J., Reichenberg, A., Henschker, D., Beck, E., Jomaa, H., Schlitzer, M. *Arch. Pharm.* **2005**, *338*, 305-314.

### **1.3.3. OBJECTIVES: DESIGN OF NEW INHIBITORS FOR DXR**

Increased efforts in antimalarial drug discovery are urgently needed. The goal must be to develop safe, affordable and effective new drugs to counter the spread of malaria parasites that are resistant to existing agents.

The research presented in this thesis is part of a larger multidisciplinary project with the common objective of developing new antimalarials that interfere with the DOXP pathway.

The scope of this thesis is to discover new inhibitors of DXR with the potential to be developed through subsequent research into a new medication for malaria. Fosmidomycin will be used as lead and the main objective is the synthesis of a series of analogues in order to expand the structure-activity relationship (SAR) of fosmidomycin analogues as DXR inhibitors.

Our investigations will be described in five chapters.

- Chapter 2.2.1 describes the synthesis of analogues characterized by modifications of the *N*-hydroxyformamido group (Figure 1.37).

In the next three chapters, modifications on the propyl chain are described (Figure 1.37).

- The synthesis of an entirely new class of fosmidomycin analogues possessing differently substituted phenyl moieties in  $\alpha$ -position of the phosphonate is described in chapter 2.3.1
- Chapter 2.3.2 discusses the synthesis of a series of analogues featuring restricted conformational mobility.
- To expand the structure-activity relationship of fosmidomycin and FR900098, analogues were synthesized in which the methylene group in  $\beta$ -position of the phosphonate moiety was replaced by an oxygen atom. The synthesis of

analogues that combine this  $\beta$ -oxa or a  $\gamma$ -oxa modification with a hydroxamic acid moiety are reported in chapter 2.3.3.

- In chapter 2.4 ensuing studies involving the synthesis of analogues that mimic the transition state are described.

The biological activities of the synthesized compounds are described in chapter 3.

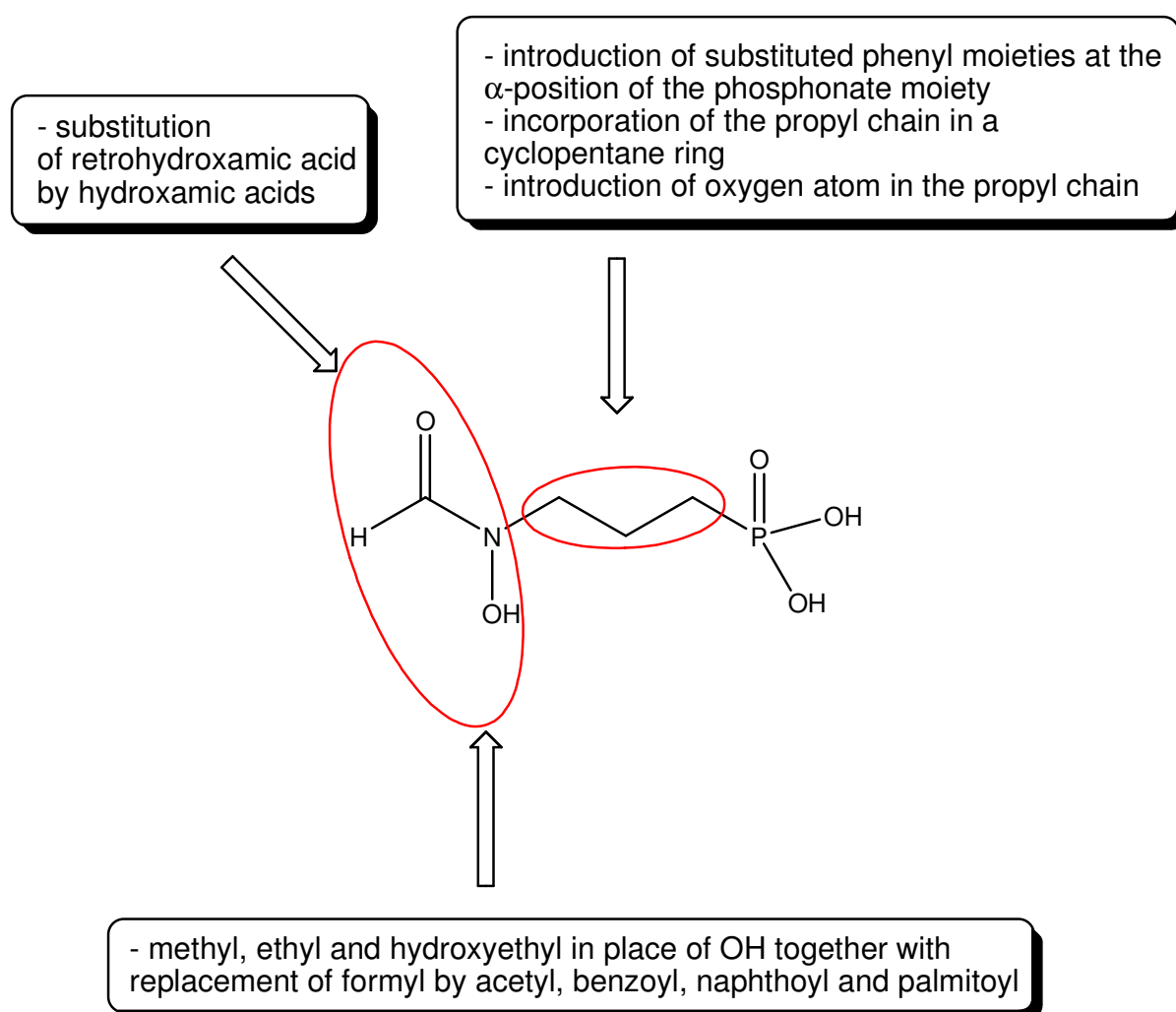


Figure 1.37: Overview of the planned modifications on fosmidomycin.

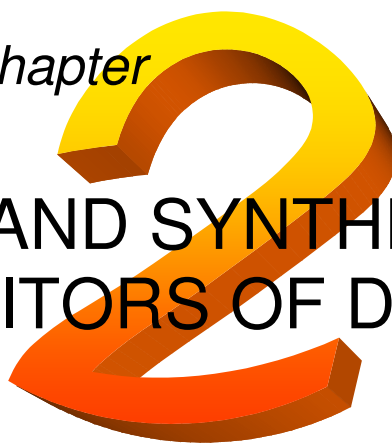






*Chapter*

# DESIGN AND SYNTHESIS OF INHIBITORS OF DXR





## **2. DESIGN AND SYNTHESIS OF INHIBITORS OF DXR**

After introducing shortly the phosphonate group (2.1.) we will discuss our design and synthesis work in three chapters:

- 2.2. Modifications of the hydroxamide moiety
- 2.3. Modifications of the propyl chain
- 2.4. Synthesis of a transition state analogue

### **2.1. BRIEF INTRODUCTION ON PHOSPHONATE CHEMISTRY**

#### **2.1.1. SYNTHESIS OF THE PHOSPHONATE MOIETY**

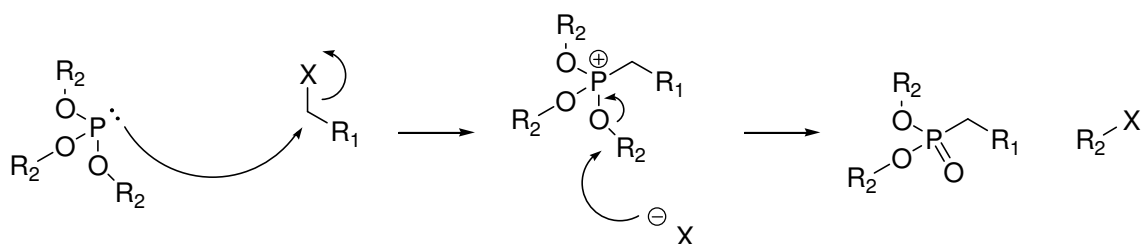
Since the phosphonic acid group is important in the binding of fosmidomycin to DXR, the introduction of the C–P bond is an essential issue in the synthesis of metabolically stable analogues. Many papers and reviews deal with the synthesis of phosphonic acids.<sup>228</sup> Two primary challenges associated with the synthesis of phosphonic acids are the formation of the C–P bond and the cleavage of the protective groups. Four major reaction types to form a C–P bond can be considered.

##### **2.1.1.1. THE MICHAELIS-ARBUSOV REACTION**

The Michaelis-Arbusov synthesis is the reaction of a trialkyl phosphite with an alkyl halide under elevated temperature with the formation of a dialkyl phosphonic ester. In this reaction a quaternary alkoxyphosphonium intermediate is formed, followed by a dealkylation accompanied by valency expansion of phosphorus to form a P=O bond. This latter process which represents a valence shell expansion of phosphorus from 8 to 10 electrons is made possible by the availability of vacant 3d orbitals.

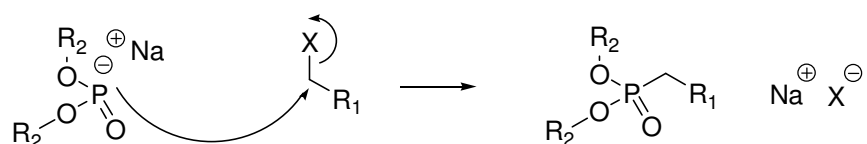
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<sup>228</sup> (a) Engel, R. *Chem. Rev.* **1977**, 77, 349-367. (b) Fields, S. C. *Tetrahedron* **1999**, 55, 12237-12273. (c) Enders, D., Saint-Dizier, A., Lannou, M.-I., Lenzen, A. *Eur. J. Org. Chem.* **2006**, 29-49.



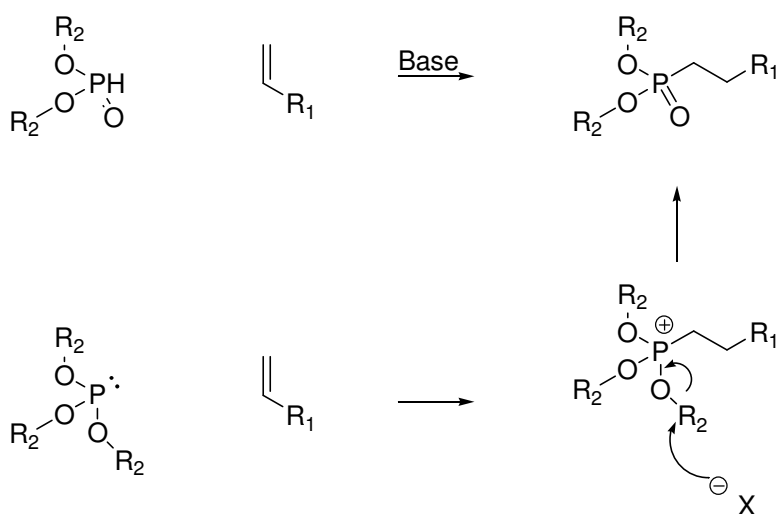
#### 2.1.1.2. THE MICHAELIS-BECKER REACTION

The Michaelis-Becker synthesis involves reaction of the alkali salt of a dialkylphosphite with an alkyl halide and the formation of the corresponding dialkyl phosphonic ester.



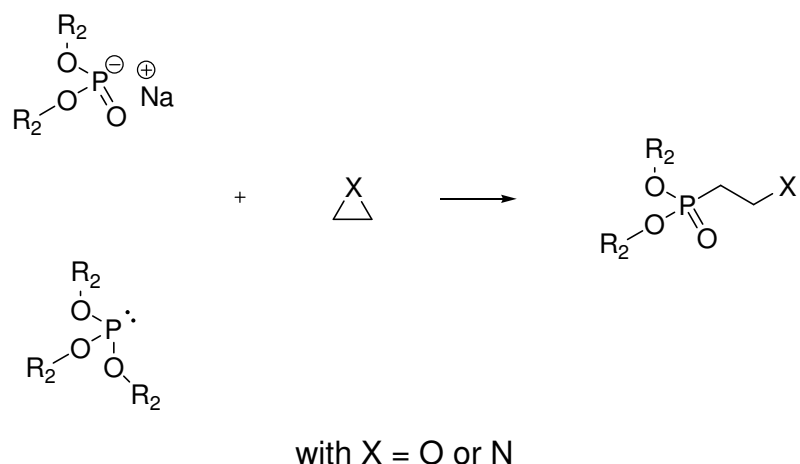
#### 2.1.1.3. A MICHAEL ADDITION OF ALKYLPHOSPHITE TO AN ACTIVATED DOUBLE BOND

The addition of a phosphorus nucleophile to an acceptor-substituted alkene or alkyne, such as an  $\alpha,\beta$ -unsaturated aldehyde or ketone, results in the corresponding dialkyl phosphonic ester.



#### 2.1.1.4. THE ADDITION OF ALKYLPHOSPHITE TO AN AZIRIDINE OR EPOXIDE

Another possibility to obtain a C–P bond is the opening of an aziridine or epoxide with trialkyl phosphite or the alkali salt of a dialkyl phosphite.



#### 2.1.2. CLEAVAGE OF THE PHOSPHONATE ESTERS

The cleavage of phosphonic acid dialkyl esters can be achieved by reflux in concentrated HCl. These harsh conditions often result in complex reaction mixtures and poor yields. A mild alternative is the transesterification with bromotrimethylsilane (TMSBr) or iodotrimethylsilane (TMSI) into the corresponding bistrimethylsilyl diester of the phosphonic acid.<sup>229</sup> This can subsequently be hydrolysed upon reaction with  $\text{H}_2\text{O}$ . An other alternative is the use of dibenzyl phosphite. The resulting dibenzyl phosphonate ester can be cleaved by hydrogenolysis.

#### 2.1.3. PROPERTIES OF FREE PHOSPHONIC ACIDS

Phosphonic acids are rather strong acids, with a higher acidity than carboxylic acids, but lower than similar sulfonic acids. In general the  $\text{pK}_\text{a}$ 's are between 2.4 and

<sup>229</sup> (a) McKenna, C. E., Schmidhauser, J. C. S. *Chem. Comm.* **1979**, 739. (b) McKenna, C. E., Higa, M. T., Cheung, N. H., McKenna, M.-C. *Tetrahedron Lett.* **1977**, 2, 155-158.

2.9 and between 7.7 and 9, respectively. The negative charge of the phosphonate anion is spread over two oxygen atoms by mesomery.

As expected, electron withdrawing groups on the  $\alpha$ -position of the phosphonate raise the acidity. The first pKa of methylphosphonic acid is 2.38 and lowers to 1.63 in trichloromethylphosphonic acid.<sup>230</sup>

The phosphonic acid group is strongly polar. Derivatives with a short carbon skeleton are highly water soluble. In organic solvents marked intermolecular hydrogen bonding takes place.

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<sup>230</sup> Quin, L. D., *A guide to organophosphorus chemistry*, Wiley Interscience (2000).

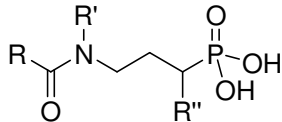
## 2.2. MODIFICATIONS OF THE HYDROXAMIDE MOIETY

### 2.2.1. MODIFICATIONS ON NITROGEN

#### 2.2.1.1. RATIONALE

At the start of this project we envisaged the synthesis of fosmidomycin analogues with a modified *N*-hydroxyformamide moiety. These analogues **2.1-2.9** are shown in Table 2.1.

Table 2.1: Overview of fosmidomycin analogues.

			
	<b>R</b>	<b>R'</b>	<b>R''</b>
<b>fosmidomycin (1.83)</b>	H	OH	H
<b>FR900098 (1.84)</b>	CH <sub>3</sub>	OH	H
<b>2.1</b>	CH <sub>3</sub>	CH <sub>3</sub>	H
<b>2.2</b>	CH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	H
<b>2.3</b>	H	CH <sub>3</sub>	H
<b>2.4</b>	H	CH <sub>2</sub> CH <sub>3</sub>	H
<b>2.5</b>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>
<b>2.6</b>	CH <sub>3</sub>	(CH <sub>2</sub> ) <sub>2</sub> OH	H
<b>2.7</b>	Ph	(CH <sub>2</sub> ) <sub>2</sub> OH	H
<b>2.8</b>	1-naphtyl	(CH <sub>2</sub> ) <sub>2</sub> OH	H
<b>2.9</b>	C <sub>15</sub> H <sub>31</sub>	(CH <sub>2</sub> ) <sub>2</sub> OH	H

First, we explored the possibility to replace the OH substituent on the nitrogen by a methyl, ethyl or hydroxyethyl group. The methyl and ethyl substituents in compounds **2.1-2.4** were introduced because in the transition state of the enzymatic conversion of DOXP there is also a methyl function on C2, which corresponds with the nitrogen in fosmidomycin and FR900098 after superposition (Figure 1.29). The

hydroxyethyl moiety on the nitrogen in compound **2.6** was introduced to investigate the effect of an extended distance between the hydroxyl function and the nitrogen.

Compounds **2.7-2.9** represent analogous compounds with more lipophilic acyl groups. Although the efficacy of fosmidomycin for the treatment of acute uncomplicated malaria was recently proven in several clinical trials, relatively high doses of fosmidomycin were required to achieve the desired results. This is partly due to the highly polar nature of this molecule. Therefore, we addressed the question whether it might be possible to obtain more lipophilic DXR inhibitors by replacing the formyl or acetyl residue of fosmidomycin or FR900098 by sterically more demanding residues.

The choice of altered acyl moieties in compounds **2.7**, **2.8** and **2.9** was based on unpublished biological results. The same is true for the introduction of a methyl in the  $\alpha$ -position of the phosphonate (**2.5**).<sup>231</sup>

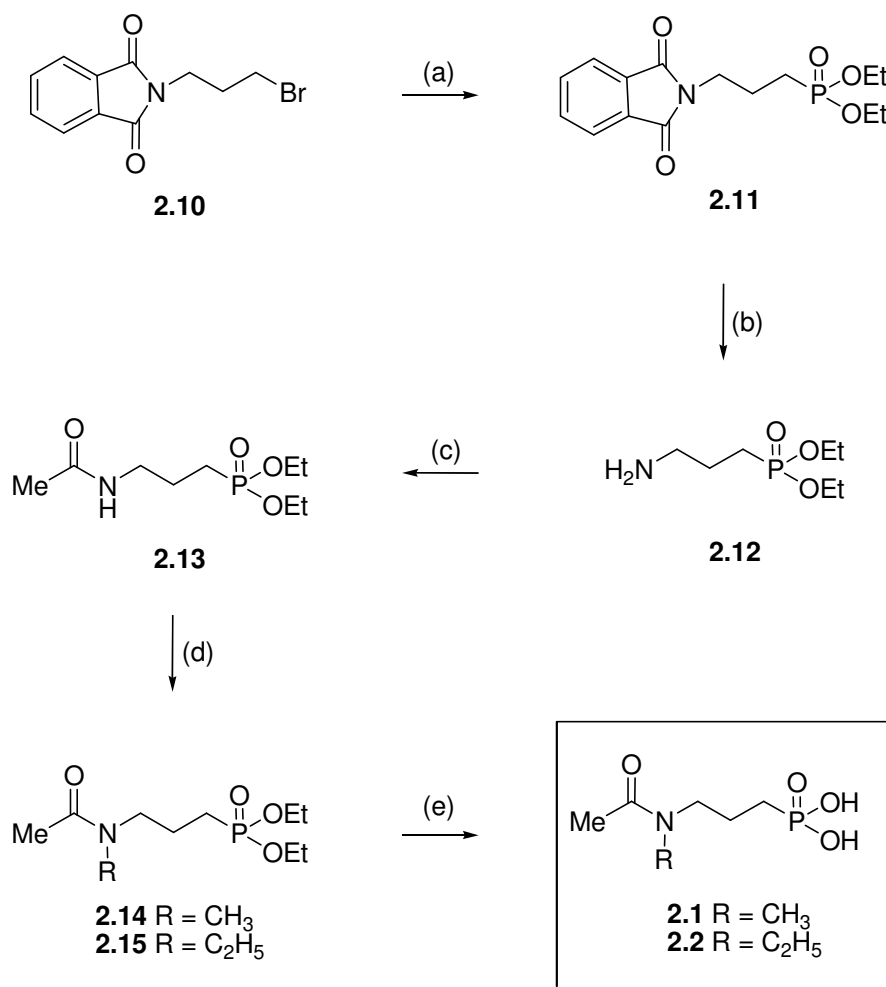
#### 2.2.1.2. SYNTHESIS

The *N*-methyl- and *N*-ethylacetamide derivatives **2.1** and **2.2** were synthesized as shown in Scheme 2.1 starting from *N*-(3-bromopropyl)phthalimide (**2.10**).<sup>232</sup> A Michaelis-Arbusov reaction with an excess of triethyl phosphite under reflux, followed by treatment of **2.11** with hydrazine monohydrate yielded diethyl 3-aminopropylphosphonate (**2.12**). After purification by column chromatography, amine **2.12** was acetylated with acetyl chloride. The resulting amide **2.13** was deprotonated with sodium hydride and alkylated with iodomethane or iodoethane to give **2.14** and **2.15**, respectively. Deprotection of the phosphonate esters to give the desired phosphonates **2.1** and **2.2** was accomplished with TMSI or TMSBr. Generally TMSI gave better yields and shorter reaction times.

<sup>231</sup> Wiesner, J., Jomaa, H. *unpublished results*.

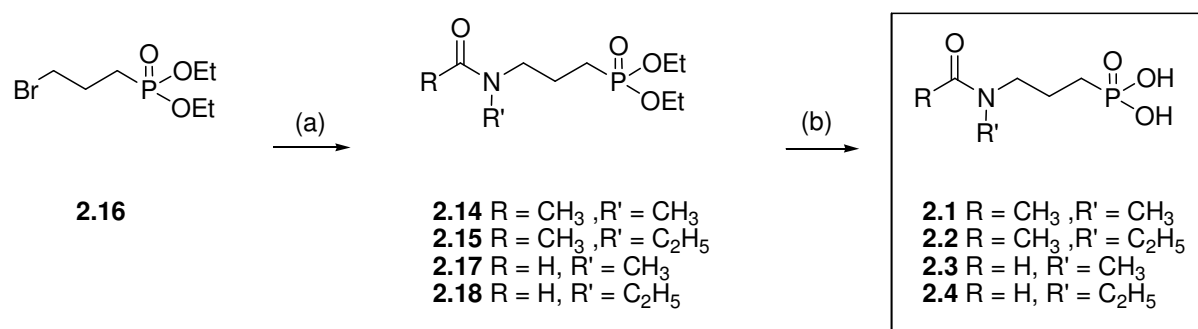
<sup>232</sup> Gali, H., Prabhu, K. R., Karra, S. R., Katti, K. V. *J. Org. Chem.* **2000**, 65, 676-680.





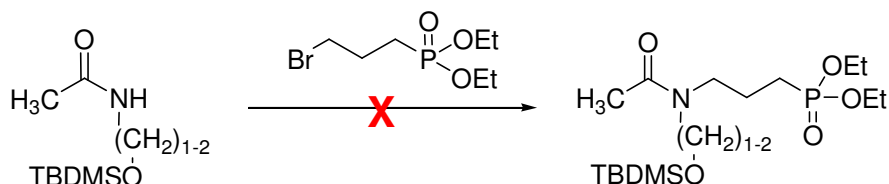
Scheme 2.1: Reagents and conditions (a) triethyl phosphite, reflux, 12 h (98%), (b) NH<sub>2</sub>NH<sub>2</sub>·H<sub>2</sub>O, EtOH, reflux, overnight (94%), (c) acetyl chloride, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, rt, 3 h (82 %), (d) MeI or EtI, NaH, THF, rt, overnight (72% and 67% respectively), (e) TMSI, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h (100 %).

The formyl analogues **2.3** and **2.4** were prepared by nucleophilic attack of the commercially available *N*-methylformamide or *N*-ethylformamide on diethyl (3-bromopropyl)phosphonate (**2.16**) after deprotonation with sodium hydride (Scheme 2.2). The yields for these reactions were 52% and 72%, respectively. This method was also successfully applied using *N*-methylacetamide or *N*-ethylacetamide to synthesize products **2.1** and **2.2**, thereby providing a more efficient method.



Scheme 2.2: Reagents and conditions (a) *N*-methylformamide, *N*-methylacetamide, *N*-ethylformamide or *N*-ethylacetamide, NaH, DMF, rt, 16 h (52-72%), (b) TMSI,  $\text{CH}_2\text{Cl}_2$ , rt, 2 h (100 %).

An attempt to prepare the hydroxyethyl and hydroxymethyl analogues in the same way as the formyl analogues **2.3** and **2.4**, using TBDMS protected *N*-hydroxyethyl- and *N*-hydroxymethylacetamide failed (Scheme 2.3). The preparation of the hydroxymethyl analogue by reaction of amide **2.13** with paraformaldehyde and anhydrous potassium carbonate in dioxane was also unsuccessful.



Scheme 2.3: Reagents and conditions: NaH, DMF, overnight.

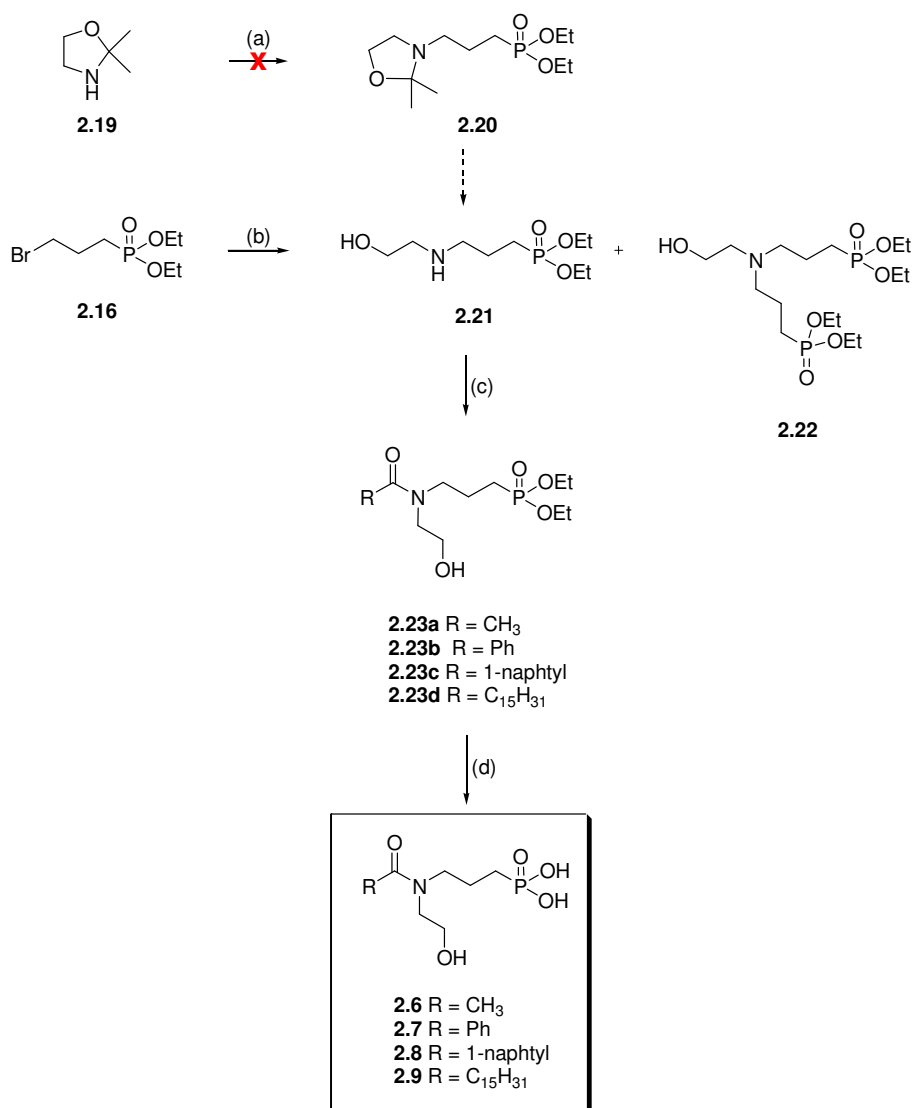
An attempt to react 2,2-dimethyl-1,3-oxazolidine **2.19**, which was prepared according to a literature procedure,<sup>233</sup> with diethyl (3-bromopropyl)phosphonate (**2.16**) was not successful either (Scheme 2.4). Although only activated bromides have been used for such alkylation reactions,<sup>234</sup> we succeeded in preparing the hydroxyethyl analogues **2.6-2.9** by selective nucleophilic attack of the amino group of ethanolamine to diethyl (3-bromopropyl)phosphonate (**2.16**) to yield compound **2.21**

<sup>233</sup> Bergmann, E. D. *Chem Rev.* **1953**, 53, 309-352.

<sup>234</sup> Falb, E., Bechor, Y., Nudelman, A., Hassner, A., Albeck, A., Gottlieb, H. E. *J. Org. Chem.* **1999**, 64, 498-506.

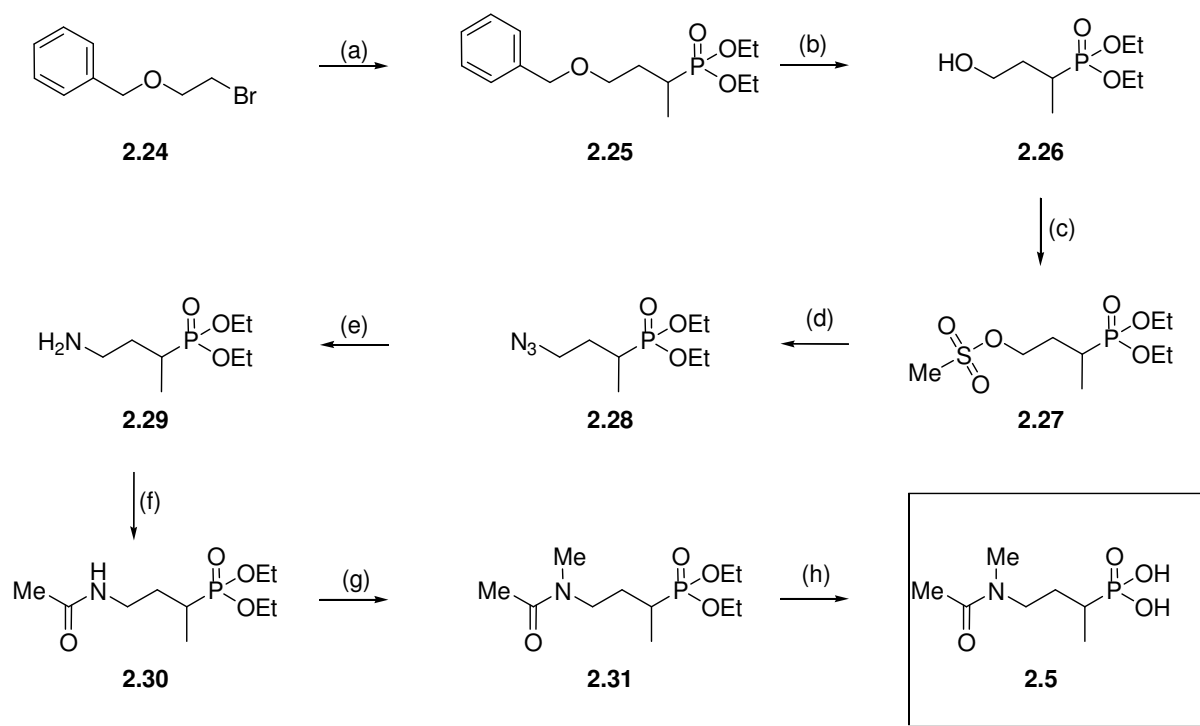
in 68% yield. Characterization by NMR of the side products revealed further reaction of the NH group with diethyl (3-bromopropyl)phosphonate (**2.16**) towards compound **2.22**. The use of an excess of 5 equivalents of ethanolamine allowed to reduce this side reaction.

Compound **2.21** was subsequently acylated with palmitoyl-, benzoyl-, 1-naphthoyl- and acetyl chloride under Schotten-Baumann conditions to achieve selective acylation of the amine in 70-80% yield. Final deprotection of **2.23** afforded the target compounds **2.6-2.9**.



Scheme 2.4: Reagents and conditions (a) diethyl (3-bromopropyl)phosphonate, Et<sub>3</sub>N, THF, 0 °C→rt, 1.5 h, (b) ethanolamine, K<sub>2</sub>CO<sub>3</sub>, acetonitrile, rt, 10 h (68%), (c) acetyl chloride, palmitoyl chloride, benzoyl chloride or 1-naphthoyl chloride, Schotten-Baumann conditions (1:1 THF:50% NaOAc), rt, 3 h (70%), (d) TMSI, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h (100%).

For the synthesis of the  $\alpha$ -methyl analogue **2.5** we first tried a similar approach as outlined in Scheme 2.1, i.e. deprotonation of an  $\alpha$  proton of diethyl ethylphosphonate with *n*BuLi and nucleophilic attack on *N*-(2-bromoethyl)phthalimide. Due to purification problems we had to look for an alternative approach. A successful attempt (Scheme 2.5) started from benzyl 2-bromoethyl ether (**2.24**) which was subjected to a reaction with diethyl ethylphosphonate in the presence of *n*BuLi to afford phosphonate **2.25**. Benzyl deprotection of **2.25** by catalytic hydrogenation gave **2.26**. Alcohol **2.26** was converted to the corresponding mesylate (**2.27**), which was displaced by an azido anion. Azide **2.28** was reduced with triphenylphosphine in pyridine, followed by addition of  $\text{NH}_4\text{OH}$ , to give amine **2.29**. This amine was further acetylated with acetyl chloride and alkylated with iodomethane to obtain compound **2.31** in an overall yield of 5%.



Scheme 2.5: Reagents and conditions (a) diethyl ethylphosphonate, *n*BuLi, -78 °C, 3 h (28%), (b)  $\text{H}_2$ , Pd/C, MeOH, rt, 5 h (83%), (c) methanesulfonyl chloride, pyridine, DMF, 0 °C, 20 h (100%), (d)  $\text{NaN}_3$ , DMF, 95 °C, 1.5 h (56%), (e) triphenylphosphine, pyridine,  $\text{NH}_4\text{OH}$ , 3 h (63%), (f) acetyl chloride, pyridine,  $\text{CH}_2\text{Cl}_2$ , rt, 2 h (86%), (g) MeI, NaH, THF, rt, overnight (25%) (h) TMSI,  $\text{CH}_2\text{Cl}_2$ , rt, 2 h (100%).

## 2.2.2. MODIFICATION OF THE RETROHYDROXAMIC ACID

### 2.2.2.1. INTRODUCTION

Two types of fosmidomycin/FR900098 analogues in which the hydroxyamide moiety is part of cyclic system were considered (Figure 2.1). First we explored the possibility to replace the *N*-hydroxyacetamide by a 2-substituted 1,2-oxazinan-3-one (**2.38**), which originates from connecting the terminal methyl group of FR900098 with the oxygen atom of the hydroxylamine part via two methylene units.

The 4-substituted 1-hydroxyazetidin-2-one **2.42b** is obtained by connecting the terminal methyl group of FR900098 with the carbon in  $\gamma$ -position of the phosphonate. We also planned to prepare **2.42a** with a shorter linker between the hydroxyazetidinone and the phosphonate moiety.

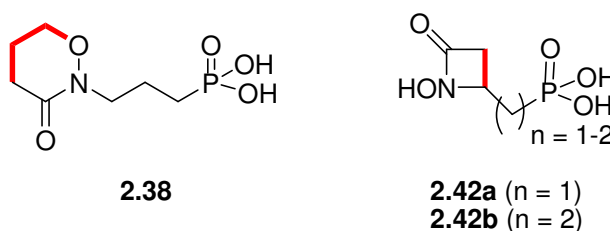


Figure 2.1: Structures of compounds **2.38** and **2.42**.

### 2.2.2.2. SYNTHESIS

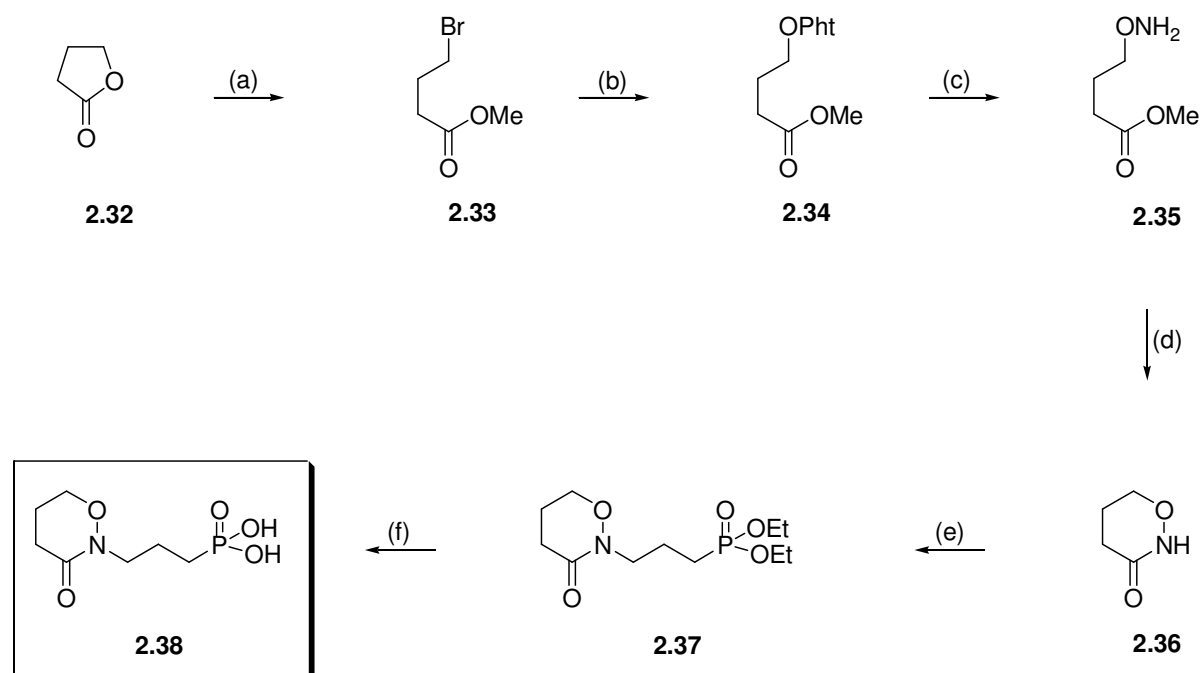
The synthesis of compounds **2.38** is shown in Scheme 2.6. 1,2-Oxazinan-3-one (**2.36**) was prepared by a method adapted from Wolfe and coworkers.<sup>235</sup> It started from butyrolactone (**2.32**), which was heated at 75 °C with a 45% solution of hydrogen bromide in acetic acid followed by treatment with methanol to give methyl  $\gamma$ -bromobutyrate **2.33**. The bromide was displaced by *N*-hydroxyphthalimide to give compound **2.34**. The aminoxy group in **2.34** was deprotected upon treatment with methylhydrazine for 1.5 h at -10 °C. Deprotection with hydrazine under reflux was too drastic since it caused hydrolysis of the methyl ester. The cyclization of **2.35** was

<sup>235</sup> Wolfe, S., Wilson, M.-C., Cheng, M.-H., Shustov, G. V., Akuche, C. I. *Can. J. Chem.* **2003**, *81*, 937-960.

achieved with trimethylaluminium in THF. A nucleophilic substitution of diethyl bromopropylphosphonate with **2.36** using Et<sub>3</sub>N in THF failed due to insolubility of the oxazinone. It also failed with potassium fluoride-alumina in DMF (Table 2.2). More effective in this case was the treatment of **2.36** with potassium carbonate in acetone. The resulting phosphonate **2.37** was deprotected with TMSBr in CH<sub>2</sub>Cl<sub>2</sub> to yield **2.38** that was further purified via reversed phase HPLC.

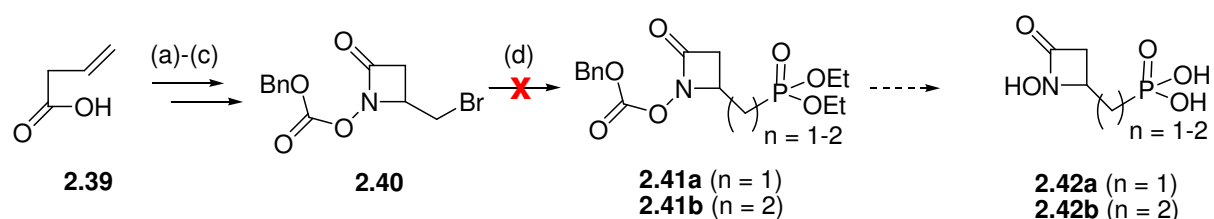
Table 2.2: Overview of reaction conditions tested to transform **2.36** to **2.37**.

Solvent	Base	Temp.	Yield
dry THF	Et <sub>3</sub> N (1.1 eq.)	rt	No reaction
dry THF	Et <sub>3</sub> N (1.1 eq.)	70 °C (reflux)	No reaction
dry DMF	KF-Al <sub>2</sub> O <sub>3</sub> (40% wt)	rt	No reaction
acetone	K <sub>2</sub> CO <sub>3</sub> (2 eq.)	60 °C (reflux)	43%



Scheme 2.6: Reagents en conditions (a) 45% HBr-HOAc, MeOH, 75 °C, 4 h (53%), (b) *N*-hydroxyphthalimide, Et<sub>3</sub>N, MeCN, reflux, 3 h (71%), (c) MeNHNH<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 1.5 h (100%), (d) AlMe<sub>3</sub>, toluene, reflux, 2 h (20%), (e) diethyl (3-bromopropyl)phosphonate, K<sub>2</sub>CO<sub>3</sub> in acetone, reflux, 1 h (43%), (f) TMSBr, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h (36%).

Our attempt toward the synthesis of *N*-hydroxy  $\beta$ -lactam **2.42** is outlined in Scheme 2.7. Bromide **2.40** was obtained by a three-step sequence as described by Rajendra and Miller.<sup>236</sup> Vinyl acetic acid was converted to an benzyl protected *O*-acylhydroxamate, which was subjected to modified Ganem's cyclization conditions to provide hydroxamate **2.40** in 39% yield.



Scheme 2.7: Reagents and conditions: (a) i.  $(\text{COCl})_2$  ii.  $\text{NH}_2\text{OH}\cdot\text{HCl}/\text{KOH}/\text{CH}_3\text{OH}$ , (b)  $\text{PhCH}_2\text{OC}(=\text{O})\text{Cl}$ , pyridine, (c)  $\text{Br}_2/\text{K}_2\text{CO}_3$ , 5-10%  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ , (d) triethyl phosphite, reflux or  $n\text{BuLi}$ ,  $\text{CH}_3\text{P}(\text{O})(\text{OEt})_2$ , THF,  $-78^\circ\text{C}$ .

Neither Michaelis-Arbusov conditions nor a reaction with diethyl methylphosphonate and  $n\text{BuLi}$  allowed to transform **2.40** into **2.41a** or **2.41b**. Complex reaction mixtures were obtained, from which we were unable to isolate the desired product.

<sup>236</sup> Rajendra, G., Miller, M. J. *Tetrahedron Lett.* **1985**, 26, 5385-5388.

## 2.3. MODIFICATIONS OF THE PROPYL CHAIN

### 2.3.1. MODIFICATIONS ON THE $\alpha$ -POSITION OF THE PHOSPHONATE GROUP

*Adapted from: "Synthesis of  $\alpha$ -substituted fosmidomycin analogues as highly potent Plasmodium falciparum growth inhibitors" Timothy Haemers, Jochen Wiesner, Sara Van Poecke, Jan Goeman, Dajana Henschker, Edwald Beck, Hassan Jomaa, Serge Van Calenbergh in Bioorg. Med. Chem. Lett. **2006**, 16, 1888-1891*

*And "Synthesis of  $\alpha$ -Aryl-Substituted and Conformationally Restricted Fosmidomycin Analogues as Promising Antimalarials" Timothy Haemers, Jochen Wiesner, Roger Busson, Hassan Jomaa, Serge Van Calenbergh in Eur. J. Org. Chem. **2006**, 17, 3856-3863.*

*This work is also the subject of a PCT patent application "Organophosphoric derivatives useful as anti-parasitic agents"*

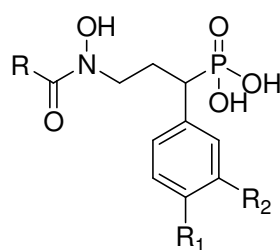
#### 2.3.1.1. INTRODUCTION AND RETROSYNTHESIS

In the 1970s, Okuhura and colleagues reported the first isolation of fosmidomycin as a structurally simple antibiotic from *Streptomyces lavendulae*. In recent years, fosmidomycin received considerable attention due to its promising antimalarial activity, and recent clinical trials conducted in Gabon and Thailand confirmed the potential of fosmidomycin as an antimalarial drug.<sup>184,185</sup> In 1998, the molecular target of fosmidomycin was discovered to be 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase.<sup>169,170</sup> This enzyme plays an essential role in the mevalonate-independent pathway for the synthesis of isoprenoids, and is absent in humans.<sup>156</sup> Fosmidomycin was found to be a potent inhibitor for the DOXP reductoisomerase (DXR) of *P. falciparum*.<sup>1</sup> After this important discovery, much attention has been focused on the chemical synthesis of fosmidomycin analogues. FR900098, the acetyl analogue of fosmidomycin, was shown to be approximately twice as active against *P. falciparum* *in vitro*, as well as in a *P. vinckei* mouse model.<sup>1</sup>



Chemical variations of fosmidomycin were mainly directed to increase the inhibitory activity against DXR or to achieve inhibitors with improved physicochemical properties. To study the structure-activity relationships, hydroxamic moiety modifications, including benzoxazolone and oxazolopyridinone functionalities (Figure 1.31), have been reported.<sup>209(b)</sup> Also, the phosphonate moiety has been altered to produce prodrugs with improved oral bioavailability.<sup>225,226,227</sup>

Surprisingly, modifications addressing the three-carbon spacer are scarce. In this chapter, we report the discovery of a series of  $\alpha$ -aryl-substituted fosmidomycin or FR900098 derivatives, **3.1** and **3.2** (Figure 3.1).



**3.1a-e** (R = H)  
**3.2a-e** (R = CH<sub>3</sub>)

- a:** R<sub>1</sub> = H; R<sub>2</sub> = H  
**b:** R<sub>1</sub> = Me; R<sub>2</sub> = H  
**c:** R<sub>1</sub> = OMe; R<sub>2</sub> = H  
**d:** R<sub>1</sub> = Cl; R<sub>2</sub> = H  
**e:** R<sub>1</sub> = Cl; R<sub>2</sub> = Cl

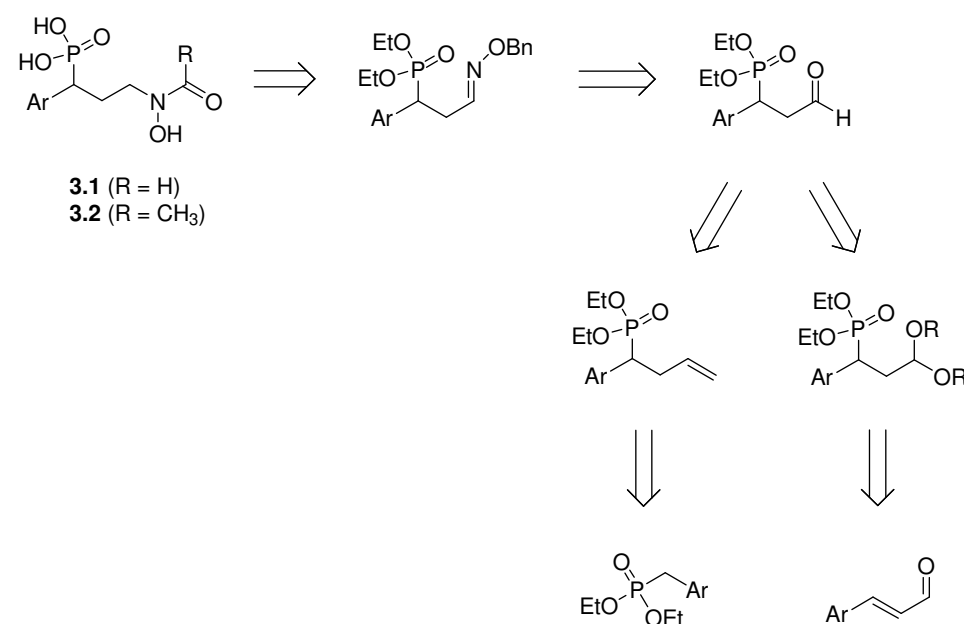
Figure 3.1: Structures of  $\alpha$ -aryl-substituted analogues of fosmidomycin and FR900098.

Although strategies to synthesise products with a C–P bond are well documented,<sup>228</sup> introducing aryl substituents in the  $\alpha$ -position of a phosphonate [resulting in a P-CH(Ar)-C motif] is quite challenging. Fosmidomycin was first synthesized in the early 1980s by Hemmi *et al.* using a Michaelis-Becker reaction.<sup>210</sup> This approach cannot be easily adapted to allow the synthesis of  $\alpha$ -substituted derivatives.

3-Aryl-substituted 3-phosphorylpropanals were anticipated to be appropriate intermediates for the synthesis of a small series of  $\alpha$ -aryl-substituted fosmidomycin analogues. Depending on the availability of the starting material, a lithiation/allylation/alkene oxidation sequence or a Michael addition will be considered for the synthesis of these intermediates (Scheme 3.1). A drawback of this strategy is

that every derivative has to be synthesized *de novo*, which does not permit preparation of an extended series of the envisaged analogues. However, when the proposed routes give the desired analogues in good overall yields, they might be valuable for scale-up purposes, e.g., to prepare a selected inhibitor for *in vivo* studies. Retrosynthetic analysis toward the synthesis of the desired  $\alpha$ -substituted fosmidomycin analogues is depicted in Scheme 3.1.

To sort out the influence of the lipophilicity and electronic properties of this phenyl moiety, substituents were introduced according to Topliss methodology.<sup>237</sup> Briefly, in this methodology an operational scheme is used to quickly identify the optimum substitution on a benzene ring for maximizing drug potency by virtue of resulting changes in hydrophobic, electronic and steric effects.



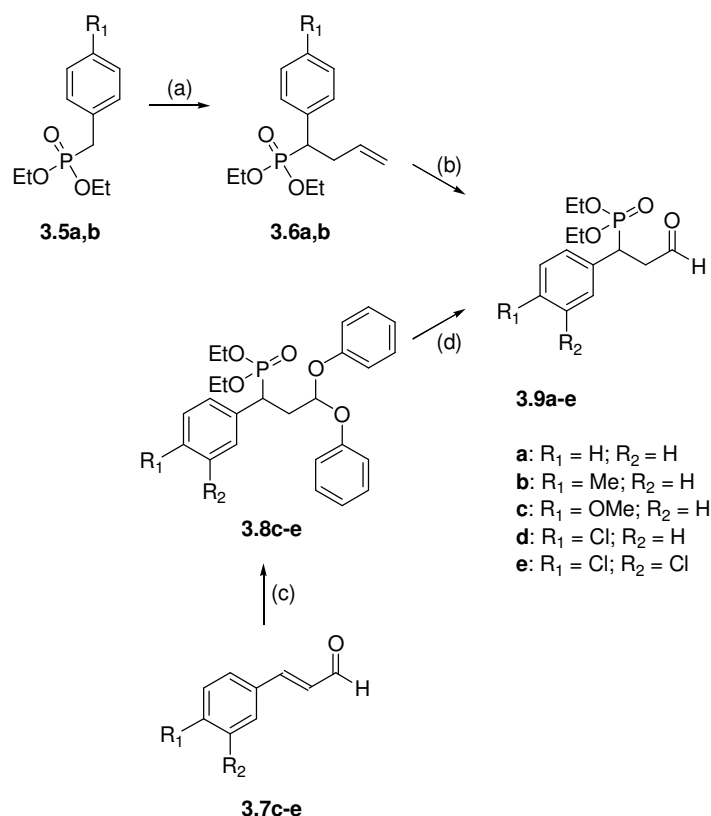
Scheme 3.1: Retrosynthetic route toward analogues **3.1** and **3.2**.

### 2.3.1.2. SYNTHESIS

Two synthetic pathways toward the aldehyde synthons were followed (Scheme 3.2). The first one started from the appropriate diethyl benzylphosphonate, which upon treatment with *n*BuLi in the presence of allyl bromide, afforded **3.6a,b** in

<sup>237</sup> a) Topliss, J. G. *J. Med. Chem.* **1972**, 15, 1006-1011, b) Topliss, J. G. *J. Med. Chem.* **1977**, 20, 463-469.

97 and 33% yields, respectively.<sup>238</sup> Oxidation of **3.6a,b** to the vicinal *cis*-diol with osmium tetroxide in the presence of 4-methylmorpholine *N*-oxide, followed by sodium periodate cleavage gave aldehydes **3.9a,b**, which could be used in the next step without further purification.



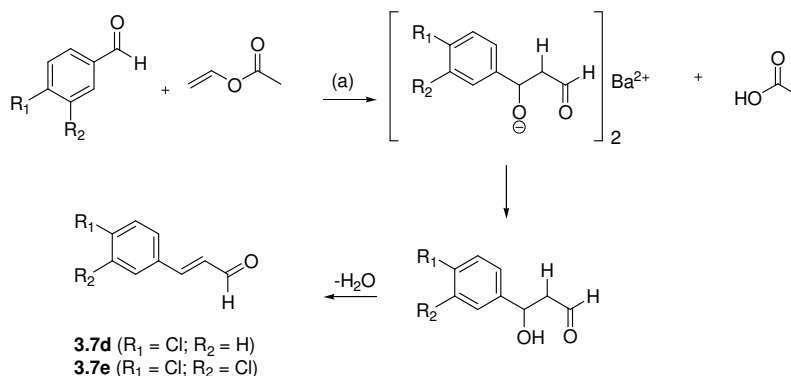
Scheme 3.2: Reagents and conditions: (a) (i) *n*BuLi, THF, -50 to -70 °C, 15 min, (ii) allyl bromide, -70 °C→reflux, 3 h (97%), (b) (i) OsO<sub>4</sub>, 4-methylmorpholine *N*-oxide, dioxane, rt, in the dark overnight, (ii) NaIO<sub>4</sub>, rt, 2 h (100%) (c) triethyl phosphite, phenol, 100 °C, 24 h (70-85%) (d) 2 N HCl, 60-70 °C, 3-4 h (76-83%).

When the proper benzylphosphonate was not commercially available, an alternative strategy to prepare the desired aldehydes was followed. A 1,4-addition of triethyl phosphite to the appropriately substituted cinnamaldehyde in the presence of phenol gave the acetals **3.8c-e** in 70-85% yield.<sup>239</sup> Subsequent deprotection of the diphenyl acetal afforded in 76-83% yield the corresponding aldehydes, which appeared stable enough to be purified by flash chromatography.

<sup>238</sup> Flitsch, W., Lubisch, W., Rosche, J. *Liebigs Ann. Chem.* **1987**, 8, 661-664.

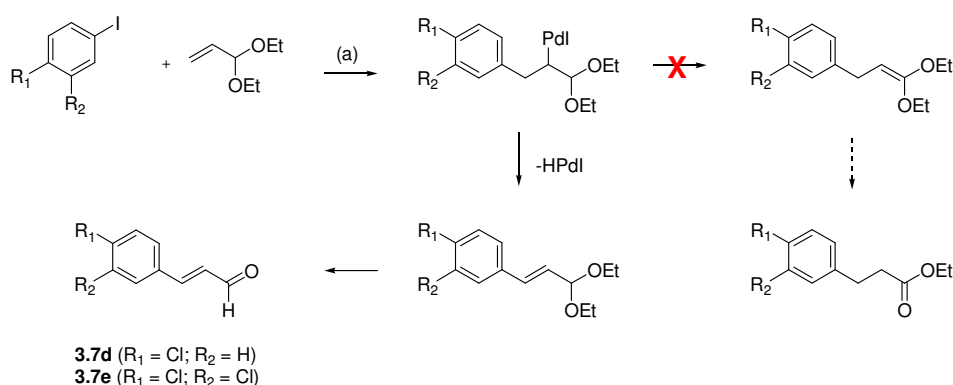
<sup>239</sup> Harvey, R. G. *Tetrahedron* **1966**, 22, 2561-2573.

If necessary, substituted cinnamaldehydes were synthesized. A reaction with vinyl acetate and the corresponding aldehydes in the presence of  $\text{Ba}(\text{OH})_2$  (Scheme 3.3)<sup>240</sup> resulted in complex reaction mixtures and low yields (< 20%).



Scheme 3.3: Reagents and conditions: (a)  $\text{Ba}(\text{OH})_2$ , THF, 65 °C, 10 h (17-20%).

In our hands a palladium-catalyzed synthesis from acrolein diethyl acetal and the corresponding aryl iodide was more efficient (yield 58-74%).<sup>241</sup> The reaction takes place in the presence of  $\text{Pd}(\text{OAc})_2$ ,  $n\text{Bu}_4\text{NOAc}$ ,  $\text{K}_2\text{CO}_3$ , KCl and DMF at 90 °C, followed by the addition of 2 N HCl. Under these modified Heck conditions the cinnamaldehyde is selectively formed and not the propanoate ester (Scheme 3.4). Only the (*E*)-isomer was obtained, as deduced from the large coupling (16 Hz) between the vinylic hydrogen atoms.

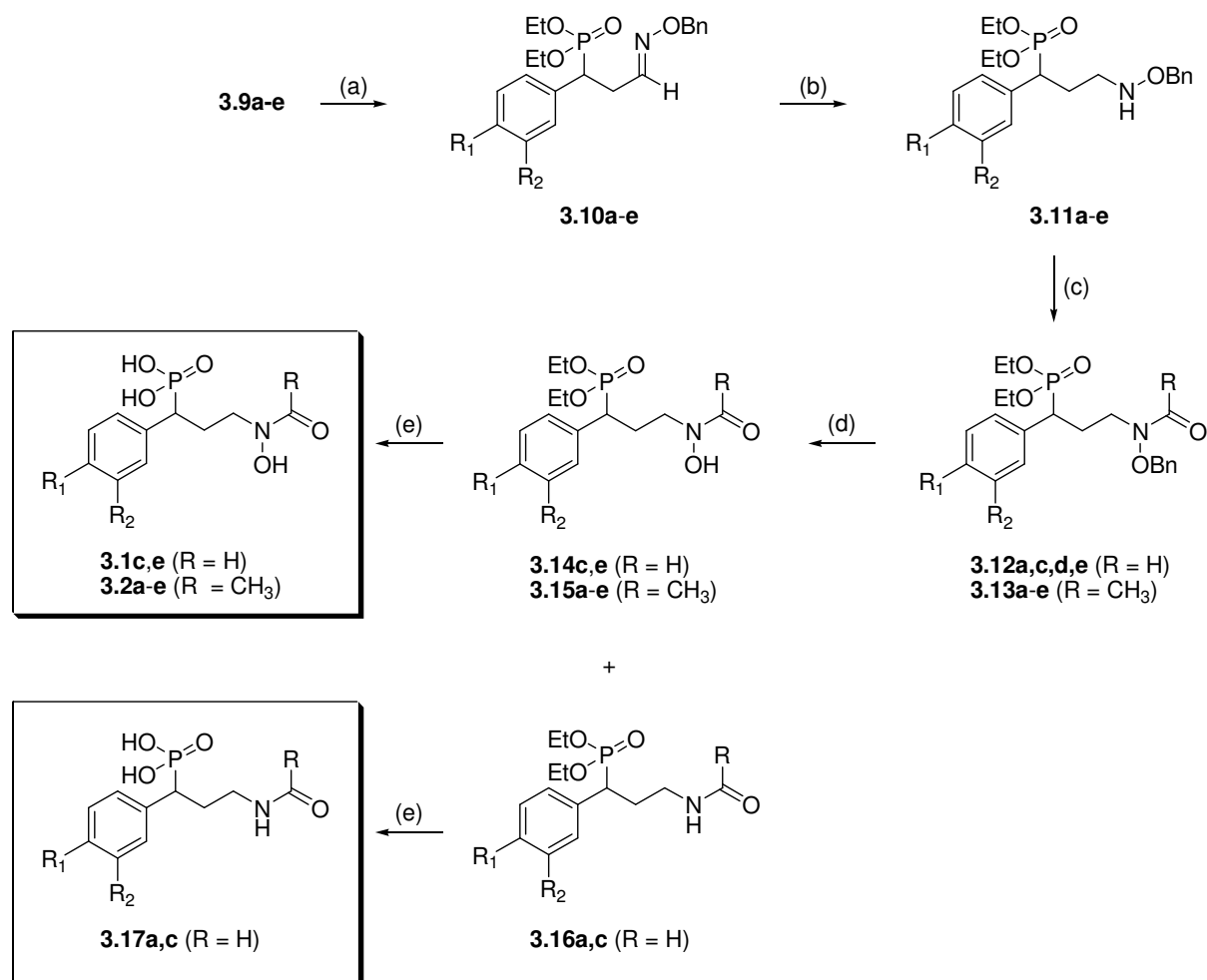


Scheme 3.4: Reagents and conditions: (a)  $\text{Pd}(\text{OAc})_2$ ,  $n\text{Bu}_4\text{NOAc}$ ,  $\text{K}_2\text{CO}_3$ , KCl, DMF, 90 °C, 1.5 h (58-74%).

<sup>240</sup> Mahata, P. K., Barun, O., Ila, H., Junjappa, H. *Synlett*. **2000**, 9, 1345-1347.

<sup>241</sup> Battistuzzi, G., Cacchi, S., Fabrizi, G. *Org. Lett.* **2003**, 5, 777-780.

Conversion of the appropriate aldehydes (**3.9a-e**) to the desired analogues **3.1** and **3.2** is depicted in Scheme 3.5.



Scheme 3.5: Reagents and conditions: (a) *O*-benzylhydroxylamine hydrochloride, pyridine, EtOH, rt, 1.5-6 h (67-92%), (b) NaCNBH<sub>3</sub>, MeOH, HCl, rt, 3-16 h (91-96%) (c) acetyl chloride, CH<sub>2</sub>Cl<sub>2</sub>, Et<sub>3</sub>N, 0 °C or carbonyldiimidazole, HCOOH, CH<sub>2</sub>Cl<sub>2</sub>, rt, 5 h (79-85%) (or 2-thioxothiazolidine-3-carbaldehyde, CH<sub>2</sub>Cl<sub>2</sub>, rt, 3 d (89%) for **3.12a**), (d) H<sub>2</sub>, Pd/C, MeOH, rt, 5 h (20-85%) (e) TMSBr, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h, almost quantitative.

Treatment of **3.9a-e** with *O*-benzylhydroxylamine yielded (67-92 %) oximes **3.10a-e**. <sup>13</sup>C NMR spectroscopy revealed the presence of two geometric isomers, which were reduced with sodium cyanoborohydride to produce the benzyloxyamines **3.11a-e** in 91-96 % yield. Subsequent acetylation of **3.11a-e** with acetyl chloride afforded **3.13a-e** in good yield. Different methods were investigated for the

formylation of **3.11**. Since the mixed anhydride method was unsuccessful, compound **3.11a** was formylated with 2-thioxothiazolidine-3-carbaldehyde, prepared by reaction between 2-mercaptothiazoline and formic acid using DCC as coupling agent. A drawback of this approach was the long reaction time (more than 3 days at room temperature). Consequently, **3.11c,d,e** were formylated using formic acid and 1,1'-carbonyldiimidazole in dichloromethane. This method reduced the reaction time considerably.

Benzyl deprotection by catalytic hydrogenation proved tricky, especially in the formyl series, where this reaction generally led to the formation of two reaction products. After their separation, mass spectrometry was useful in assigning these compounds as the desired product and the corresponding deoxygenated derivative, i.e. the amide. Further structural evidence for this deoxygenation was furnished by a  $^1\text{H}$  COSY NMR spectrum of the side product, which shows a strong coupling between the  $\text{NCH}_2$  protons and a heteroatom-bound proton at  $\delta = 7.04$  ppm. This coupling is normally absent in the desired products, as may be expected for such long-range  $^4J(\text{CH}_2\text{NOH})$  coupling.

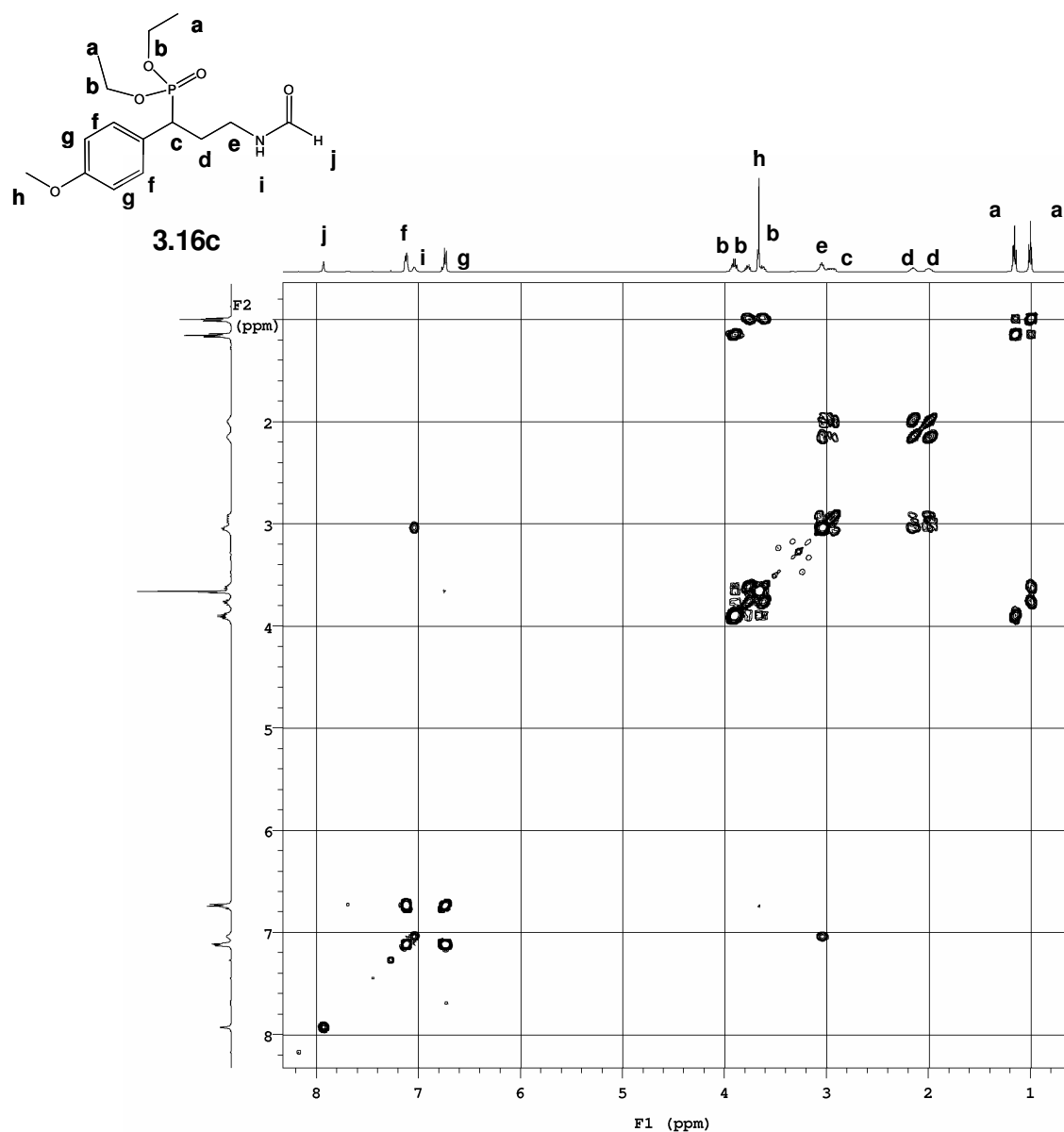
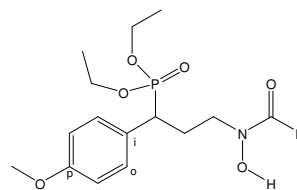


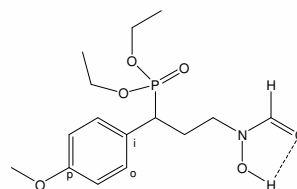
Figure 3.6:  $^1\text{H}$  COSY NMR spectrum of product **3.16c**, which shows a strong coupling between the  $\text{NCH}_2$  protons and a heteroatom-bound proton at  $\delta = 7.04$  ppm.

Also, the characteristic  $^{13}\text{C}$  NMR upfield shifts of the  $\text{N-CH}_2$  carbon signal are in agreement with the absence of the OH group on the nitrogen atom ( $\beta$ -substituent effect). Indeed, for the deoxygenated product **3.16c** the  $\text{N-CH}_2$  signal appeared at  $\delta = 35.8$  ppm, while for product **3.14c** two signals at  $\delta = 47$  and 44 ppm were found (Figure 3.7). This indicates that **3.14c** (and also **3.14e**) exists as a mixture of *syn*- and *anti*-NOH rotamers in a 2:1 ratio.

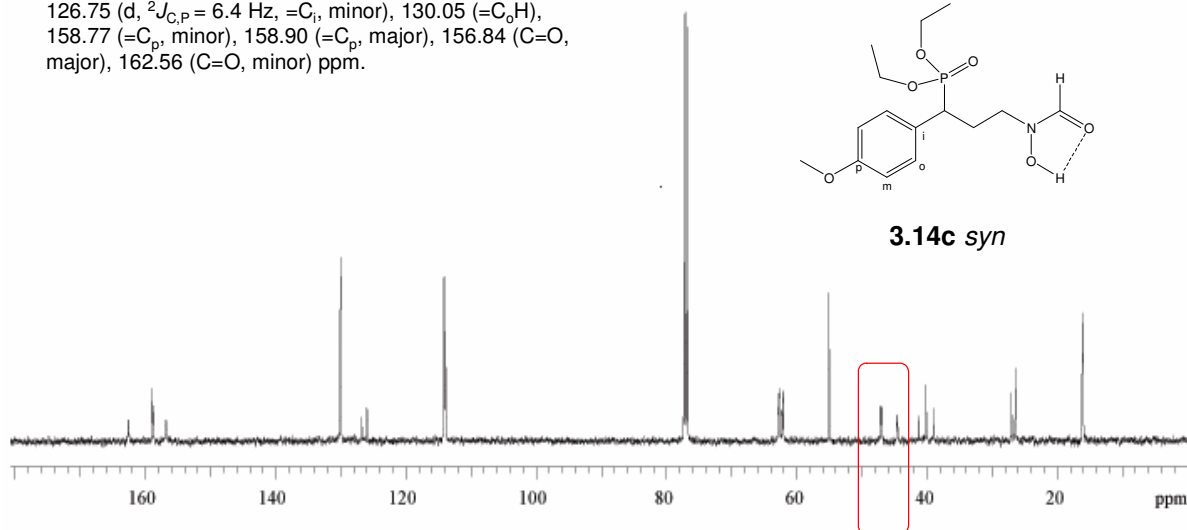
**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): d = 16.05 (d, <sup>3</sup>J<sub>C,P</sub> = 5.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.15 (d, <sup>3</sup>J<sub>C,P</sub> = 5.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 26.40 (CH<sub>2</sub>CHP, major), 27.02 (CH<sub>2</sub>CHP, minor), 39.56 (d, <sup>1</sup>J<sub>C,P</sub> = 139.2 Hz, CHP, major), 40.66 (d, <sup>1</sup>J<sub>C,P</sub> = 137.2 Hz, CHP, minor), 44.56 (d, <sup>3</sup>J<sub>C,P</sub> = 16.6 Hz, NCH<sub>2</sub>, minor); 47.05 (d, <sup>3</sup>J<sub>C,P</sub> = 16.1 Hz, NCH<sub>2</sub>, major), 55.03 (OCH<sub>3</sub>), 62.07 (d, <sup>2</sup>J<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>, major), 62.25 (d, <sup>2</sup>J<sub>C,P</sub> = 6.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>, minor), 62.69 (d, <sup>2</sup>J<sub>C,P</sub> = 6.3 Hz, OCH<sub>2</sub>CH<sub>3</sub>, major), 62.83 (d, <sup>2</sup>J<sub>C,P</sub> = 5.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>, minor), 113.89 (=C<sub>m</sub>H, minor), 114.11 (=C<sub>m</sub>H, major), 125.94 (d, <sup>2</sup>J<sub>C,P</sub> = 7.3 Hz, =C<sub>i</sub>, major), 126.75 (d, <sup>2</sup>J<sub>C,P</sub> = 6.4 Hz, =C<sub>i</sub>, minor), 130.05 (=C<sub>o</sub>H), 158.77 (=C<sub>p</sub>, minor), 158.90 (=C<sub>p</sub>, major), 156.84 (C=O, major), 162.56 (C=O, minor) ppm.



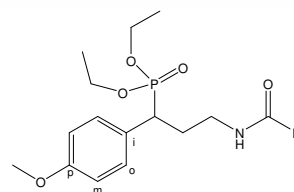
**3.14c anti**



**3.14c syn**



**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): d = 15.89 (d, <sup>3</sup>J<sub>C,P</sub> = 4.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.03 (d, <sup>3</sup>J<sub>C,P</sub> = 5.0 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 29.45 (CH<sub>2</sub>CHP), 35.80 (d, <sup>3</sup>J<sub>C,P</sub> = 16.5 Hz, NCH<sub>2</sub>), 40.89 (d, <sup>1</sup>J<sub>C,P</sub> = 116.2 Hz, CHP), 54.85 (OCH<sub>3</sub>), 61.68 (d, <sup>2</sup>J<sub>C,P</sub> = 7.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.35 (d, <sup>2</sup>J<sub>C,P</sub> = 6.7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 113.93 (d, J<sub>C,P</sub> = 2.0 Hz, =C<sub>m</sub>H), 126.61 (d, J<sub>C,P</sub> = 7.4 Hz, =C<sub>i</sub>), 129.78 (d, J<sub>C,P</sub> = 6.6 Hz, =C<sub>o</sub>H), 158.58 (d, J<sub>C,P</sub> = 2.8 Hz, =C<sub>p</sub>), 161.25 (C=O) ppm.



**3.16c**

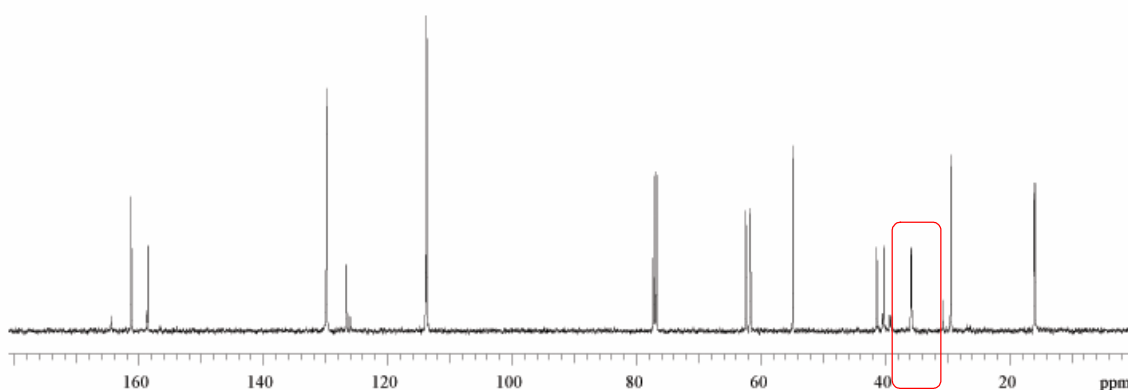


Figure 3.7: <sup>13</sup>C NMR's of **3.14c** and **3.16c**.



Compounds **3.14c,e** and **3.15a-e** were finally deprotected with 4 equivalents of TMSBr in CH<sub>2</sub>Cl<sub>2</sub> at ambient temperature to afford **3.1c,e** and **3.2a-e** after purification by reversed-phase HPLC. Although this reaction was almost quantitative, minor amounts of deacylated products were detected, probably due to small amounts of HBr in the TMSBr reagent. Compounds **3.16a,c** were deprotected in the same way, and evaluated for their activity to demonstrate the importance of the NOH group.

In a <sup>1</sup>H NMR experiment compound **3.2b** was subjected to higher temperatures to investigate the influence on the two rotamers. In table 3.1 the proton shifts at 80 °C demonstrated the coalescence of the *syn*- and *anti*-NOH rotamers. Also the <sup>13</sup>C NMR spectra confirmed this coalescence by elevated temperatures.

Unfortunately, attempts carried out at Advanced Separation Technologies Inc. (Whippany, NJ, USA) to resolve the racemate **3.1e** into its pure enantiomers using a whole range of chiral columns remained unsuccessful. We believe that the proton on C-α is too acidic to allow isolation of the pure enantiomers.

Table 3.1: comparison of <sup>1</sup>H NMR (D<sub>2</sub>O, 500 MHz) data of compound **3.2b** by two different temperatures.

	δ (ppm) by 27 °C	δ (ppm) by 80 °C
CH <sub>2</sub> N	<i>syn</i> : 3.58 & 3.38 <i>anti</i> : 3.53 & 3.46	3.55 & 3.42
CHP	<i>syn</i> : 2.85 <i>anti</i> : 3.06	2.87
C-CH <sub>2</sub> -C	<i>syn</i> : 2.35 & 2.18 <i>anti</i> : 2.47 & 2.18	2.38 & 2.17
Me-Ar	<i>syn</i> : 2.30 <i>anti</i> : 2.30	2.30
MeC=O	<i>syn</i> : 2.02 <i>anti</i> : 1.70	1.95 (broad signal)

### 2.3.2. CYCLOPENTANE FOSMIDOMYCIN ANALOGUES

*Adapted from: "Synthesis of  $\alpha$ -Aryl-Substituted and Conformationally Restricted Fosmidomycin Analogues as Promising Antimalarials" Timothy Haemers, Jochen Wiesner, Roger Busson, Hassan Jomaa, Serge Van Calenbergh in Eur. J. Org. Chem. **2006**, 17, 3856-3863.*

#### 2.3.2.1. RATIONALE

Fosmidomycin exhibits close structural similarities with the intermediate from the DXR catalyzed reaction (Figure 1.29). Both share a formyl and phosphonate (or phosphate) moiety separated by five chemical bonds. Earlier results confirmed that extension or shortening of the linker has a negative effect on the inhibitory activity.<sup>242</sup> To acquire greater understanding in the structure-activity relationship of fosmidomycin, we considered the synthesis of analogues of fosmidomycin featuring a strongly reduced mobility. Such strategy is often followed in medicinal chemistry to deduce the "active conformation" of highly flexible molecules. Conformationally restricted  $\gamma$ -aminobutyric acid (GABA) analogues, for instance, have been used to help identify three major GABA receptors. GABA is a highly flexible molecule, therefore, it can attain many low-energy conformations that bind to the different GABA receptors.<sup>243</sup> Fosmidomycin, consists of a linear highly flexible chain of three carbon atoms bearing a phosphonate functionality on one side and a hydroxyformamide functionality on the other side. Obviously, this chain can adopt a lot of conformations. One of the possibilities to reduce the number of possible conformations is restrict the free rotation around these three bonds. This can be realized via connection of the  $\alpha$ - and  $\beta$ -carbon giving rise to a three-membered ring. Indeed, by incorporating the  $\alpha$ - and  $\beta$ -carbon atoms in a cyclopropane ring, we recently demonstrated that rigidification of fosmidomycin might result in potent DXR inhibitors.<sup>213</sup> Consequently, it was thought that other conformationally restricted analogues like **3.3** and **3.4** should be investigated.

<sup>242</sup> Wiesner, J., Jomaa, H. *Unpublished results*.

<sup>243</sup> Chebib, M., Johnston, G. A. R. *Clin. Exp. Pharmacol. Physiol.* **1999**, 26, 937-940.

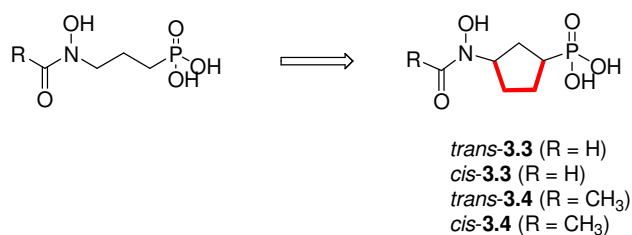
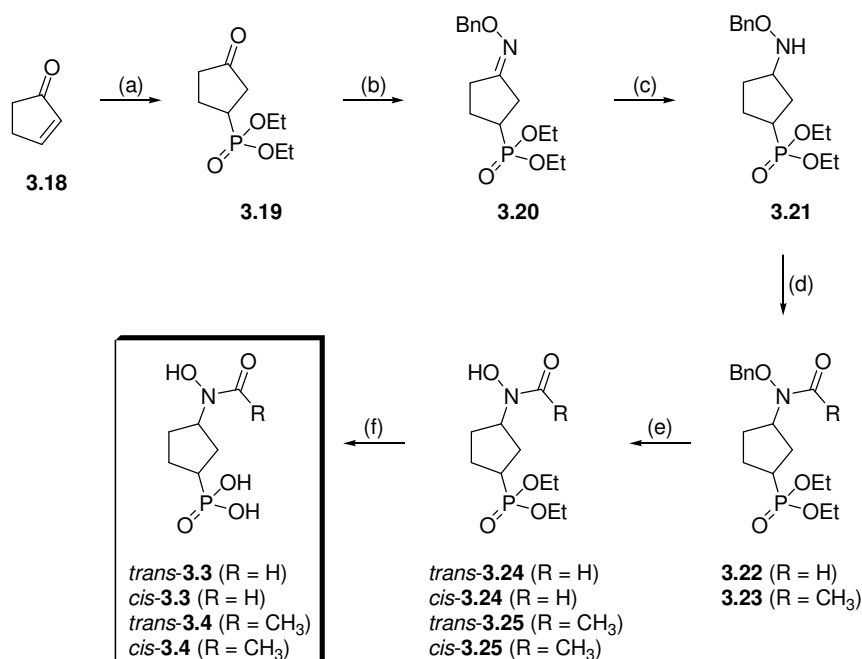


Figure 3.8: Structures of the target conformationally restricted analogues of fosmidomycin and FR900098.

### 2.3.2.2. SYNTHESIS

The global strategy employed for the synthesis of the desired cyclopentane analogues, is based on the synthesis of the  $\alpha$ -substituted analogues. We anticipated that, when applied to cyclopent-2-enone, a Michael addition could be a useful approach to prepare the envisaged fosmidomycin analogues **3.3** and **3.4**, from cyclopent-2-enone (Scheme 3.9).



Scheme 3.9: Reagents and conditions: (a) triethyl phosphite, phenol, 100 °C, 24 h (90%), (b) *O*-benzylhydroxylamine, pyridine, EtOH, rt, 6 h (90%), (c) NaCNBH<sub>3</sub>, MeOH, HCl, rt, 17 h (80%), (d) acetyl chloride, CH<sub>2</sub>Cl<sub>2</sub>, Et<sub>3</sub>N, 0 °C, 2 h (95%) or carbonyldiimidazole, HCOOH, CH<sub>2</sub>Cl<sub>2</sub>, rt, 5 h (97%), (e) H<sub>2</sub>, Pd/C, MeOH, rt, 5 h (19-46%), (f) TMSBr, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h, almost quantitative.

Indeed, Michael addition of triethyl phosphite to this cyclic  $\alpha,\beta$ -unsaturated ketone gave direct access to the diethyl 3-oxocyclopentylphosphonate **3.19**.<sup>239</sup> The remaining part of the synthesis involved the same transformations as used for the  $\alpha$ -aryl phosphonates. Separation of the diastereomeric pairs was realized after the hydrogenolysis. The *cis* and *trans* isomers were assigned by  $^1\text{H}$  NOEDIF NMR experiments: an interaction between the NOH group and the methyl groups of the phosphonate ester was observed for *cis*-**3.24** and *cis*-**3.25**, as opposed to the *trans* isomers, where such a contact was missing (Figure 3.10).

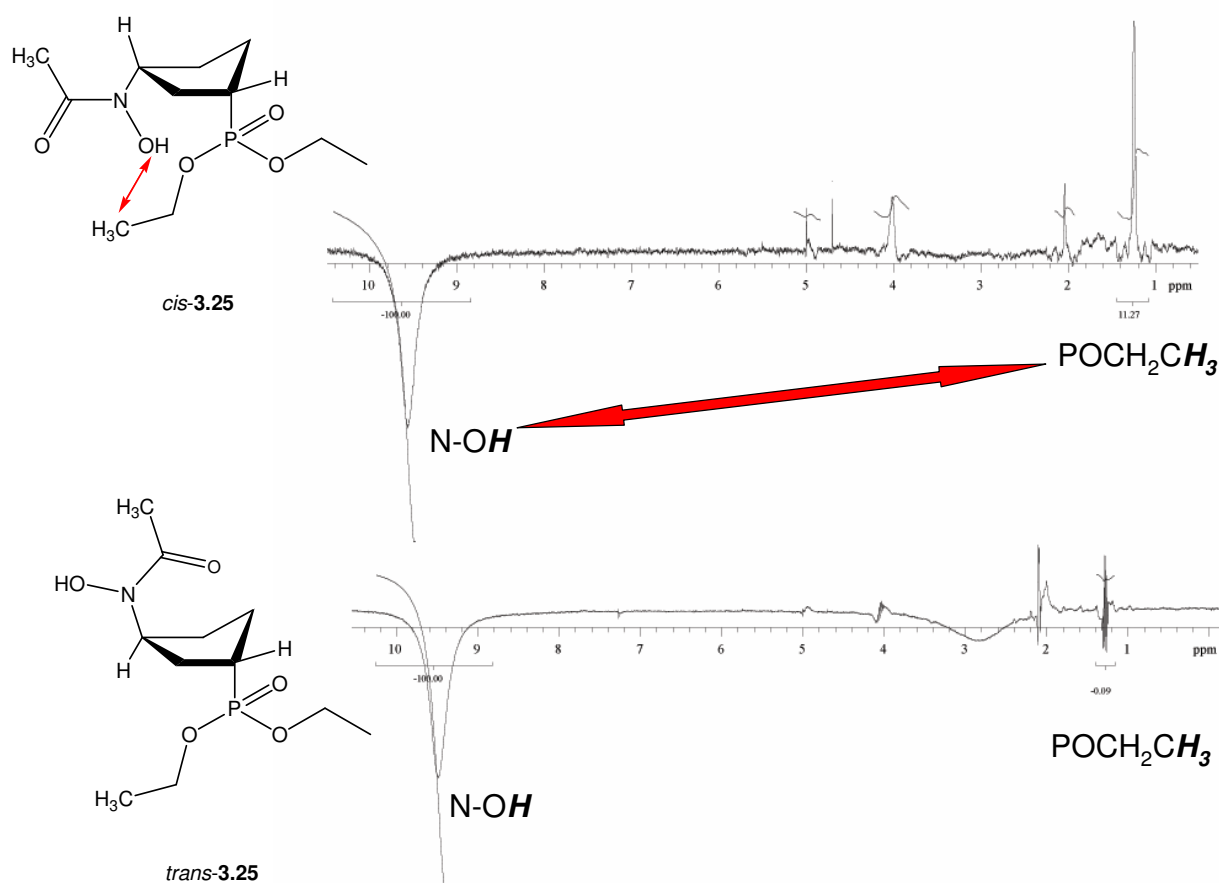


Figure 3.10:  $^1\text{H}$  NOEDIF NMR ( $\text{CDCl}_3$ , 500 MHz) after irradiation of the hydroxyl proton (NOH) of *cis*-**3.25** (interaction between  $\text{POCH}_2\text{CH}_3$  and NOH) and  $^1\text{H}$  NOEDIF NMR after irradiation of the hydroxyl proton (NOH) of *trans*-**3.25** (contact is missing between  $\text{POCH}_2\text{CH}_3$  and NOH).

The  $^{13}\text{C}$  NMR spectra of compounds **3.24** further point to the presence of a major and a minor form, probably as a result of restricted rotation in the

retrohydroxamic group, with preferential formation of the *syn* isomer due to a likely hydrogen bond between the *N*-hydroxy group and the carbonyl group.

### 2.3.3. INTRODUCTION OF AN OXYGEN ATOM IN $\beta$ - AND $\gamma$ - POSITION OF THE PHOSPHONATE FUNCTION

*Adapted from: "Synthesis of  $\beta$ - and  $\gamma$ -oxa Isosters of Fosmidomycin and FR900098 as Antimalarial Candidates" Timothy Haemers, Jochen Wiesner, Dirk Gießmann, Andreas Link, Hassan Jomaa, and Serge Van Calenbergh submitted for publication.*

#### 2.3.3.1. INTRODUCTION

1-Deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) catalyzes the conversion of 1-deoxy-D-xylulose 5-phosphate (DOXP) to 2-C-methyl-D-erythritol 4-phosphate (MEP). This conversion involves two consecutive steps (Figure 4.1). The enzyme first catalyzes an intramolecular rearrangement of DOXP into the intermediate 2-C-methyl-D-erythrose 4-phosphate. This step requires a divalent metal ion to induce a partial positive charge on the C2 carbon, allowing the C4 atom to attack it. Several studies have been reported on the mechanism of this isomerisation step.<sup>191,217,216</sup> A second step involves an NADPH-dependent reduction of the aldehyde intermediate.

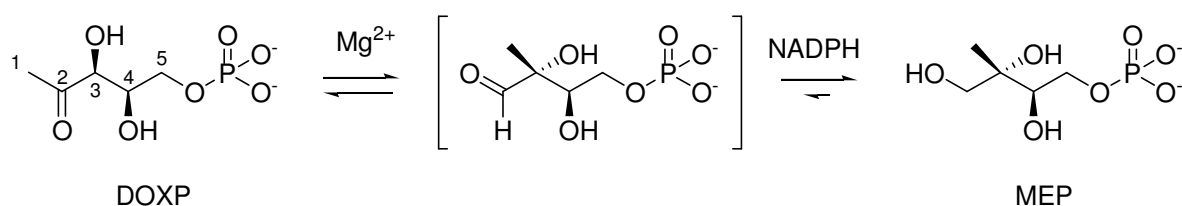


Figure 4.1: The conversion of 1-deoxy-D-xylulose 5-phosphate (DOXP) to 2-C-methyl-D-erythritol 4-phosphate (MEP) by deoxyxylulose 5-phosphate reductoisomerase (DXR).

The X-ray structural data that are available today aid in defining the structural requirements for the design of potent DXR inhibitors. A complex of DXR with

fosmidomycin with an open conformation revealed that no hydrophobic contacts are present between the three carbon spacer and the rigid part of the binding site or with the partially ordered catalytic loop.<sup>197</sup> Although, in a more recent ternary complex of DXR with fosmidomycin and NADPH, which exhibits a so-called tight-binding conformation, hydrophobic contacts were observed.<sup>198</sup> Changes of the linker, that do not prolong the linker, such as the reported aryl modification in  $\alpha$ -position, appear to target the open conformation, since a small and totally enclosed inhibitor-binding pocket is proposed.<sup>198</sup>

Recently, Proteau and coworkers described the synthesis of the phosphate analogues of fosmidomycin (**1.99**) and FR900098 (**1.100**).<sup>215</sup> The former had previously been isolated from *Pseudomonas fluorescens* PK-52 and is known as fosfoxacin.<sup>222</sup> The latter proved to be a very potent inhibitor of *Synechocystis* DXR. These results prompted us to investigate a related series of  $\beta$ - and  $\gamma$ -oxa analogues **4.1-4.6** (Chart 4.1).

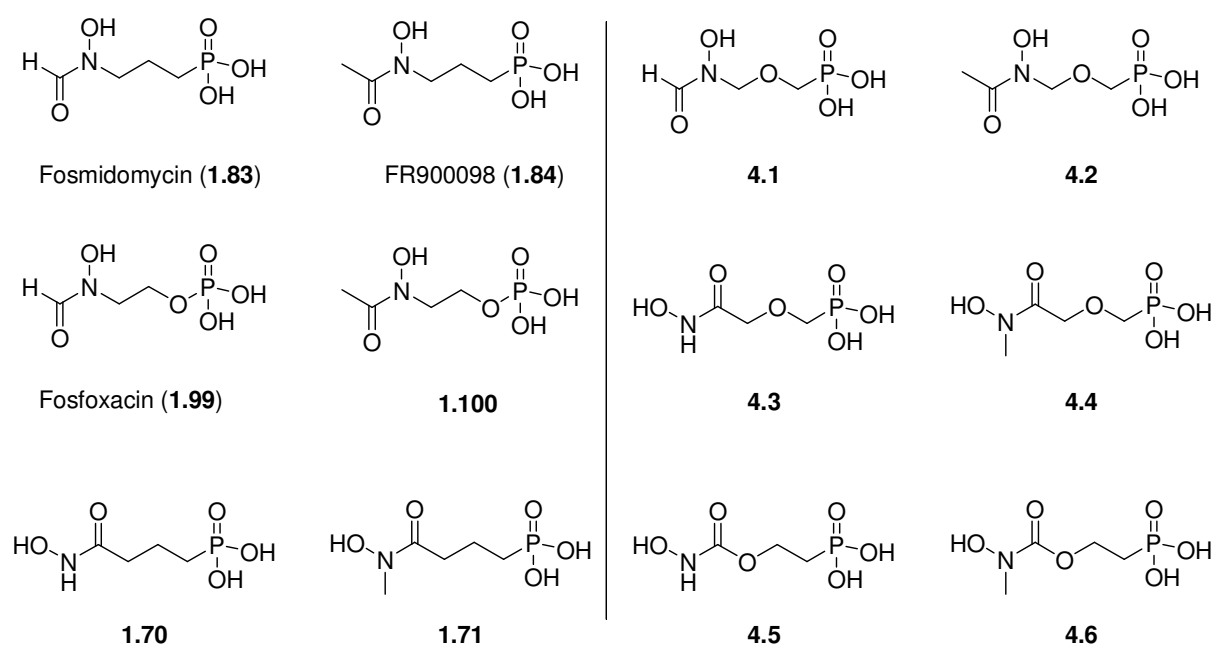


Chart 4.1: Structures of fosmidomycin, FR900098, reported (**1.99**, **1.100**, **1.70**, **1.71**) and synthesized (**4.1-4.6**) analogues.

Compounds **4.1** and **4.2** are analogues of fosmidomycin and FR900098, respectively, in which the  $\beta$ -methylene function was replaced by an oxygen atom. Since a phosphonmethoxy group has been successfully employed as a stable iso-

electronic alternative of a methyl phosphate moiety to produce antiviral nucleosides,<sup>244</sup> compounds **4.1** and **4.2** were selected as possible bioisosteres of **1.99** and **1.100**.

The bridging oxygen of the phosphate group of DOXP may be involved in interactions with amino acids in the binding site.<sup>218</sup> Since only hydrophobic contacts with the methylene linker were reported, the introduction of an oxygen atom in  $\beta$ - or  $\gamma$ -position in search for hydrogen bonding, seems less compelling. Moreover, the introduction of the electronegative oxygen atom could cause a perturbation of the electronic properties, *in casu* the deprotonation of the phosphonate group<sup>218</sup> or hydroxamate group, which becomes slightly more acidic. As a consequence of the logical decrease of the second pKa, the phosphonate could appear rather in his doubly ionised form, which is more favourable for an ideal binding. Analogues **4.3** and **4.4** combine the  $\beta$ -oxa modification with an hydroxamic acid moiety, as generally found as metal-binding group in matrix metalloproteinase inhibitors. Rohmer and coworkers reported that such metal chelating group, when replacing the *N*-hydroxyformamido moiety of fosmidomycin, as in compound **1.70** or its *N*-methyl analogue **1.71**, yielded potent inhibitors of DXR which were also capable to inhibit *E. coli* growth.<sup>206</sup> Poulter and Walker reported an analogue of DOXP in which the acetyl was replaced by a hydroxamic acid, but it did not inhibit the enzyme,<sup>221</sup> probably because the molecule did not longer fit in the confined volume of the active site, as a consequence of its one-atom longer chain. This is consistent with the most recently reported X-ray structure.<sup>198</sup>

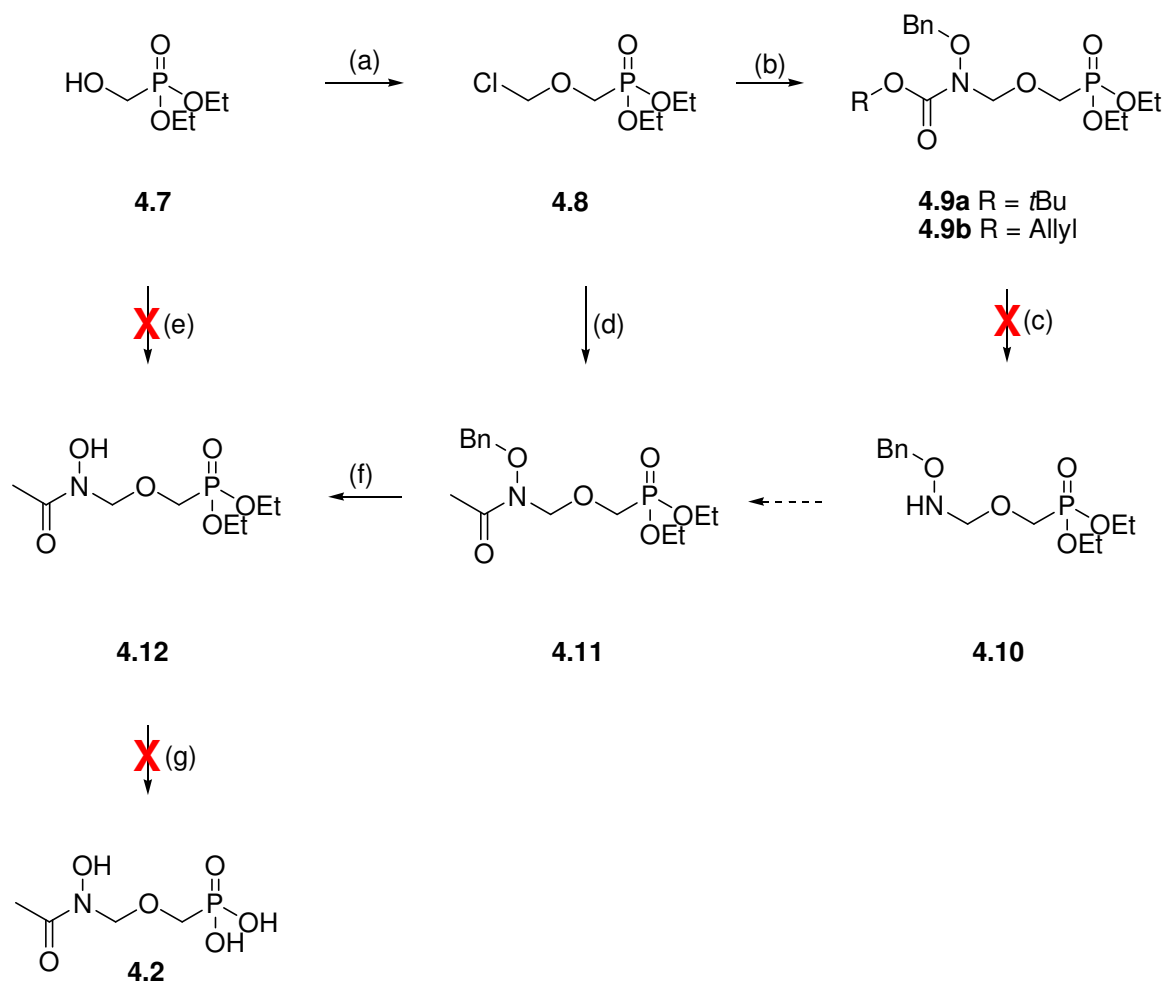
Analogues **4.5** and **4.6**, which are constitutional isomers of **4.3** and **4.4**, were also synthesized. They combine a hydroxamic acid moiety with an oxygen in  $\gamma$ -position that could possibly mimic the C4 hydroxyl group present in the substrate. The C4 hydroxyl group is involved in hydrogen bonding with Glu152, Asn227 and Lys228.<sup>198</sup>

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<sup>244</sup> De Clercq, E., Holy. A. *Nat. Rev. Drug Discov.* **2005**, *4*, 928-940.

### 2.3.3.2. SYNTHESIS OF $\beta$ - AND $\gamma$ -OXA ISOSTERS OF FOSMIDOMYCIN AND FR900098

#### 2.3.3.2.1 Synthesis of compounds **4.1** and **4.2**



Scheme 4.1: Reagents and conditions: (a) 1,3,5-trioxane, 1,2-dichloroethane, HCl, 5 °C→25 °C, 16 h, (b) *t*-butyl *N*-benzyloxycarbamate or allyl *N*-benzyloxycarbamate, NaH, DMF, rt, 2 h, (c) see text, (d) *N*-(benzyloxy)acetamide (**4.15**), NaH, DMF, rt, overnight, (e) see text, (f) H<sub>2</sub>, Pd/C, MeOH, rt, 3 h, (g) TMSBr, CH<sub>2</sub>Cl<sub>2</sub>, rt.

A first attempt (step e in Scheme 4.1) towards the synthesis of analogue **4.2** started from the commercially available diethyl hydroxymethylphosphonate (**4.7**) using an intermolecular reaction between **4.7**, acetohydroxamic acid and paraformaldehyde.<sup>245</sup> Because water must be removed from the reaction mixture,

<sup>245</sup> Geffken, D. *Arch. Pharm.* **1985**, 318, 895-902.



dried molecular sieves or a Dean Stark apparatus were both separately evaluated. Unfortunately these attempts were not successful and no product was obtained.

Treatment of **4.7** with 1,3,5-trioxane, in the presence HCl gas, afforded (chloromethoxy)methylphosphonate (**4.8**) (58%), as reported by Kim and coworkers.<sup>246</sup> This chloromethyl ether was used in the next step without purification, albeit it was only 67% pure. An attempt to couple *N*-hydroxyacetamide directly to **4.8** with NaH in DMF resulted in complex mixtures. Coupling of **4.8** with *t*-butyl *N*-benzyloxycarbamate in the presence of NaH resulted in **4.9a**, but in the following deprotection step with trifluoroacetic acid or 4N HCl in dioxane, only compound **4.7** was obtained. To explore an alternative *N*-protective group that is resistant to NaH, *N*-Allyloxycarbonyl-*O*-benzylhydroxylamine was prepared from commercially available *O*-benzylhydroxylamine hydrochloride and allyl chloroformate. The coupling resulted in poor yield (16%). Unfortunately, deprotection of the allyl protecting group using Pd(Ph<sub>3</sub>P)<sub>4</sub> and dimedone in THF also failed.<sup>247</sup>

In both cases we were not able to isolate the desired product from the complex reaction mixtures. We also tried to trap **4.10** by adding acetic anhydride to the deprotection reaction, but this also resulted in an unresolvable reaction mixture. Hence, **4.8** was coupled to *N*-(benzyloxy)acetamide (**4.15**) which was prepared from acetylation of *O*-benzylhydroxylamine hydrochloride. A complex reaction mixture was obtained from which **4.11** could be isolated in a modest yield of 23%.

Catalytic hydrogenation of **4.11** gave the desired product **4.12** besides significant amounts of the corresponding amide. This side reaction was confirmed by the <sup>1</sup>H-NMR spectrum in which the γ-protons were split by the amide proton.

Deprotection of **4.12** to **4.2** with TMSBr failed due to cleavage and formation of **4.7**, which was confirmed by LC-MS and NMR. This was not fully unexpected since TMSI cleaved the same C–O bond in a similar uracil derivative, but TMSCl left the same bond intact.<sup>248</sup> In view of the problems encountered to produce analogue **4.2**, it became clear that other protecting groups of the phosphonate were required.

Scheme 4.2 depicts our successful approach to the synthesis of analogues **4.1** and **4.2**. Retrohydroxamic acids **4.14** and **4.15**, obtained by acylation of *O*-benzylhydroxylamine **4.13**, were treated with paraformaldehyde and potassium *tert*-

<sup>246</sup> Kim, C. U., Misco, P. F., Martin, J. C. *Nucleosides and Nucleotides* **1990**, 9, 579-585.

<sup>247</sup> Kunz, H., Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1984**, 23, 436-437.

<sup>248</sup> (a) Kundu, N. G., Hertzberg, R. P., Schmitz, S. A., Khatri, S. G., Kunda, N. G., Sikdar, S. *J. Chem. Soc. Perkin Trans 1* **1985**, 7, 1295-1300. (b) Kundu, N. G., Khatri, S. G. *Synthesis* **1985**, 3, 232-324.

butoxide to give the hydroxymethylated products **4.16** and **4.17**, which were subsequently converted to the chlorides **4.18** and **4.19** as reported by Zhu *et al.*<sup>249</sup> Since attempts to crystallize the chlorides from ethyl acetate led to hydrolysis to alcohols **4.16** and **4.17**, crude **4.18** and **4.19** were directly used in the Williamson synthesis with **4.21**. Addition of one equivalent of NaH did not prove effective to increase the moderate yield. Compound **4.21** was prepared by a neat reaction with dibenzyl phosphite, paraformaldehyde and triethylamine. The perbenzylated compounds **4.22** and **4.23** were finally deprotected by catalytic hydrogenation to yield the phosphonic acids **4.1** and **4.2**, which were purified by reversed phase HPLC. Since the N,O-acetal in **4.22** and **4.23** is acid sensitive, it was very important to use neutral Pd/C catalyst to prevent cleavage. Sajiki *et al.* reported a remarkable supplier dependent disparity in properties of Pd/C catalysts. Pd/C catalysts of some suppliers appeared to be acidic enough to cleave triethylsilylethers without H<sub>2</sub>.<sup>250</sup>  $\beta$ -Oxa analogue **4.2** proved to be stable in H<sub>2</sub>O for more than two weeks, as revealed by <sup>1</sup>H-NMR.

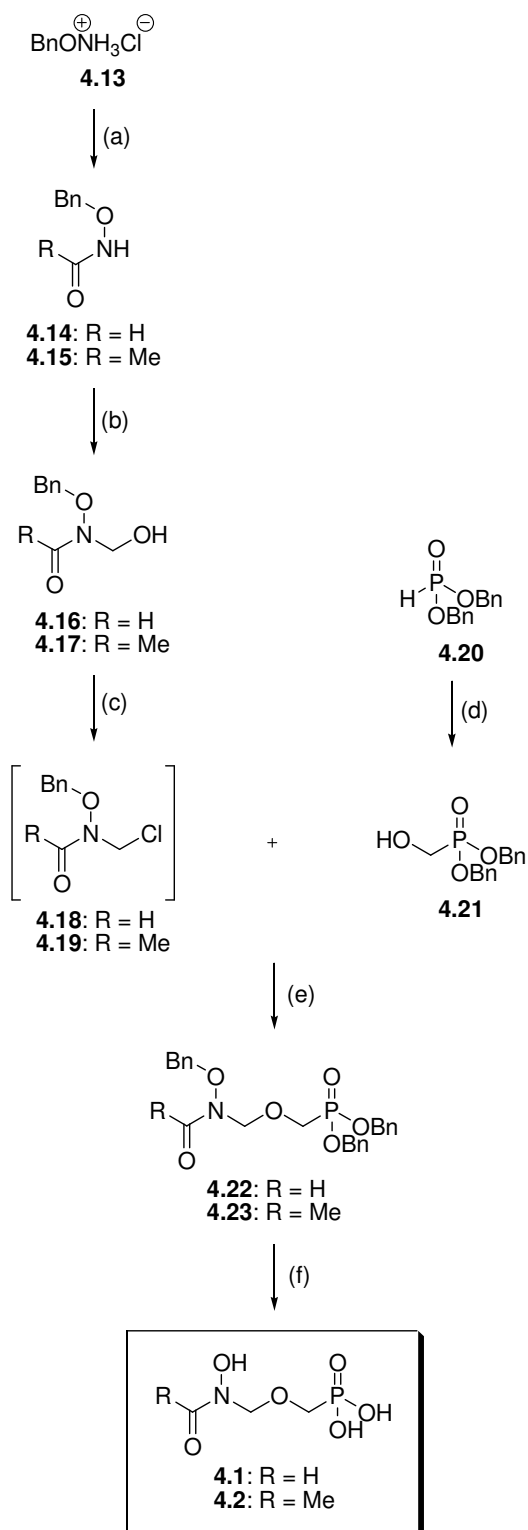
In view of the promising biological results (see chapter 3) of the  $\alpha$ -aryl analogues of fosmidomycin, attempts were made to combine the  $\beta$ -oxa modification with an aryl group in  $\alpha$ -position (Scheme 4.3). The synthesis was based on a similar approach used to prepare **4.1** and **4.2**, but with racemic benzyl hydroxyl aryl methylphosphonates instead of **4.21**. Differently substituted hydroxyl aryl methylphosphonates were prepared from dibenzyl phosphite (**4.20**) and the appropriate aryl aldehyde in the presence of MgO.<sup>251</sup> This strategy could also give entrance to chiral  $\alpha$ -aryl- $\beta$ -oxa analogues, since the synthesis of enantiomerically pure hydroxyl aryl methylphosphonates are reported.<sup>252</sup> Unfortunately, we were unable to deprotect **4.26/4.27** neither by hydrogenolysis nor with BCl<sub>3</sub>. In both cases decomposition was observed.

<sup>249</sup> Zhu, J., Robin, S., Goasdoné, N., Goasdoné, C., Loupy, A., Galons, H. *Synlett* **1995**, 97-98.

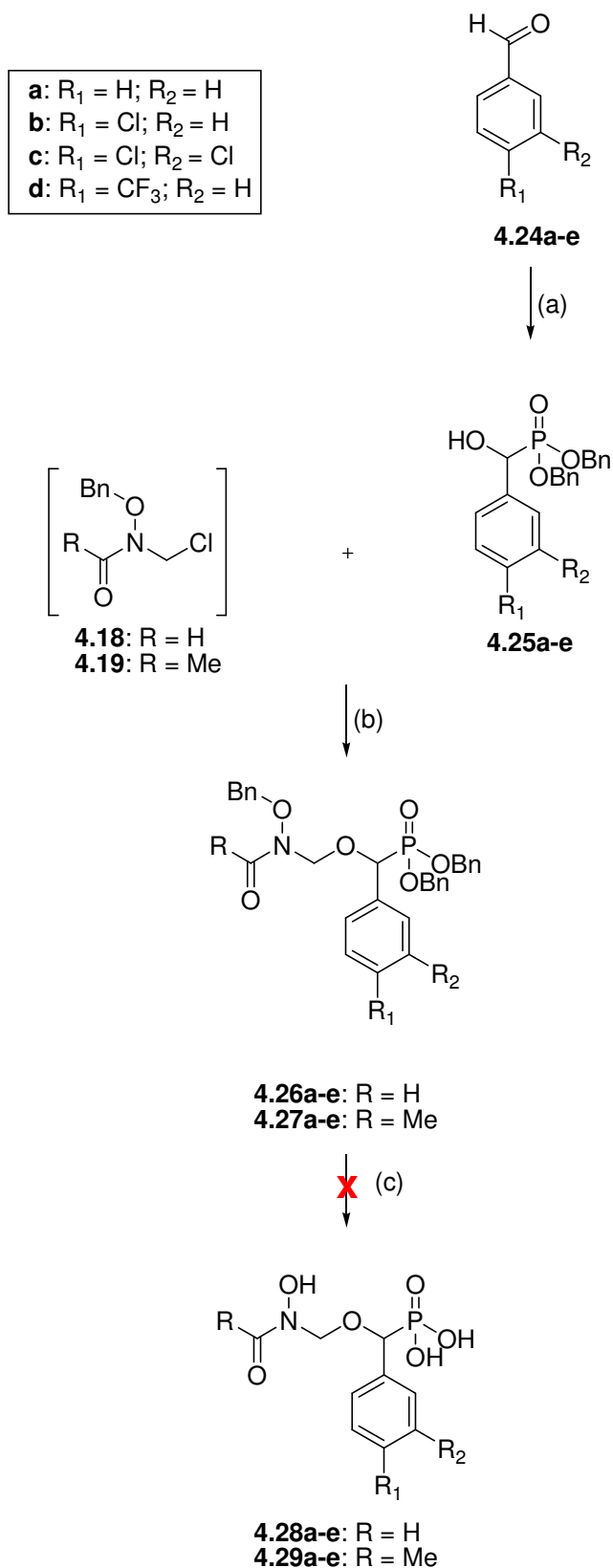
<sup>250</sup> (a) Sajiki, H., Ikawa, T., Hirota, K. *Tetrahedron Lett.* **2003**, 44, 7407-7410. (b) Ikawa, T., Sajiki, H., Hirota, K. *Tetrahedron* **2004**, 60, 6189-6195.

<sup>251</sup> Katritzky, A. R., Rogovoy, B. V., Mitrokhin, A. Y. *ARKIVOC* **2002**, 13, 17-27.

<sup>252</sup> Cermak, D. M., Du, Y., Wiemer, D. F. *J. Org. Chem.* **1999**, 64, 388-393.



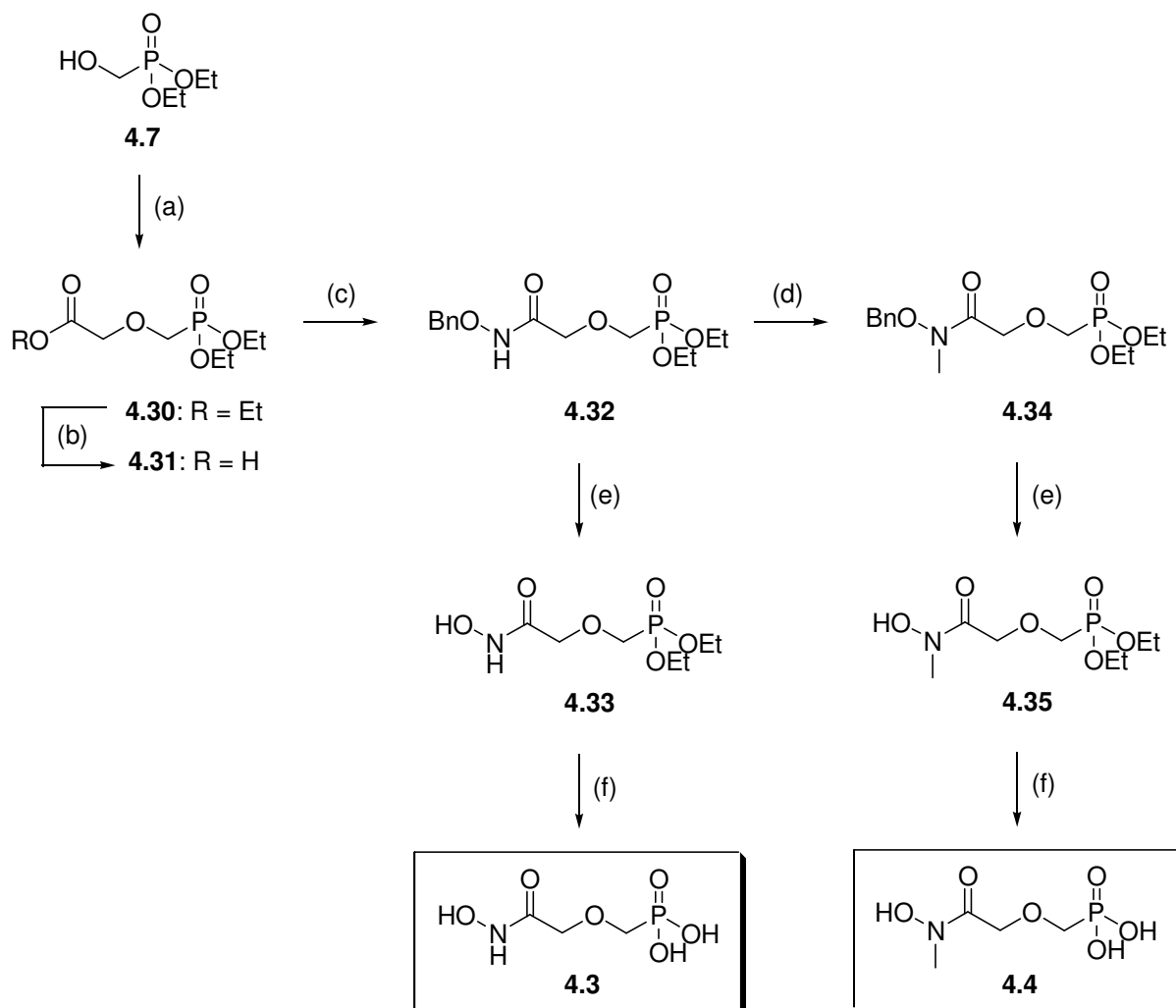
Scheme 4.2: Reagents and conditions: (a) carbonyldiimidazole, HCOOH, rt, 3 h (87%) or acetyl chloride, CH<sub>2</sub>Cl<sub>2</sub>, Et<sub>3</sub>N, 0 °C, 1.5 h (86%), (b) paraformaldehyde, *t*-BuOK, 60 °C, 4 h (79% and 48%), (c) SOCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 10 °C, 30 min (100%), (d) paraformaldehyde, Et<sub>3</sub>N, 90 °C, 12 h (56%), (e) CH<sub>2</sub>Cl<sub>2</sub>, rt, 16 h (21% and 38%) (f) H<sub>2</sub>, Pd/C, MeOH, rt, 3 h (34% and 43%).



Scheme 4.3: Reagents and conditions: (a) MgO, HP(O)(OBn)<sub>2</sub>, rt, (b) CH<sub>2</sub>Cl<sub>2</sub>, rt, (c) H<sub>2</sub>, Pd/C, MeOH, rt or BCl<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C.

### 2.3.3.2.2 Synthesis of compounds **4.3** and **4.4**

The synthesis of the hydroxamate **4.3** and its *N*-methyl analogue **4.4** is depicted in Scheme 4.4.



Scheme 4.4: Reagents and conditions: (a) ethyl bromoacetate, NaH, DMF, rt, 3 h (80%), (b) NaOH, H<sub>2</sub>O, 30 °C, 2 h (94%), (c) carbonyldiimidazole, NH<sub>2</sub>-O-Bn·HCl, Et<sub>3</sub>N, rt, 3 h (83%), (d) MeI, NaH, rt, overnight (56%), (e) H<sub>2</sub>, Pd/C, MeOH, rt, 3 h (80% and 89%), (f) TMSBr, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h (52% and 45%).

Diethyl hydroxymethylphosphonate (**4.7**) was reacted with ethyl bromoacetate in the presence of two equivalents sodium hydride in THF to give **4.30** in 80% yield. Hydrolysis of **4.30** with one equivalent of sodium hydroxide followed by neutralisation gave acid **4.31** in 94% yield. An attempt to react diethylhydroxymethyl phosphonate

directly with chloroacetic acid in the presence of two equivalents of sodium hydride resulted in a poor yield.

Several methods have been developed for the preparation of hydroxamic acids and are well documented in the literature. Hydroxamic acids have generally been synthesized in solution from nitro compounds or through reaction of *O/N*-protected hydroxylamines, such as  $\text{NH}_2\text{-O-Bn}$ , *N*-*t*-BOC-*O*-THP, *N*-*t*-BOC-*O*-TBDMS, *N,O*-bis-(phenoxycarbonyl)hydroxylamine, *N,O*-bis-(*tert*-butoxycarbonyl)hydroxylamine and *N,N,O*-tris(trimethylsilyl)hydroxylamine with activated carboxylic acids.<sup>253</sup>

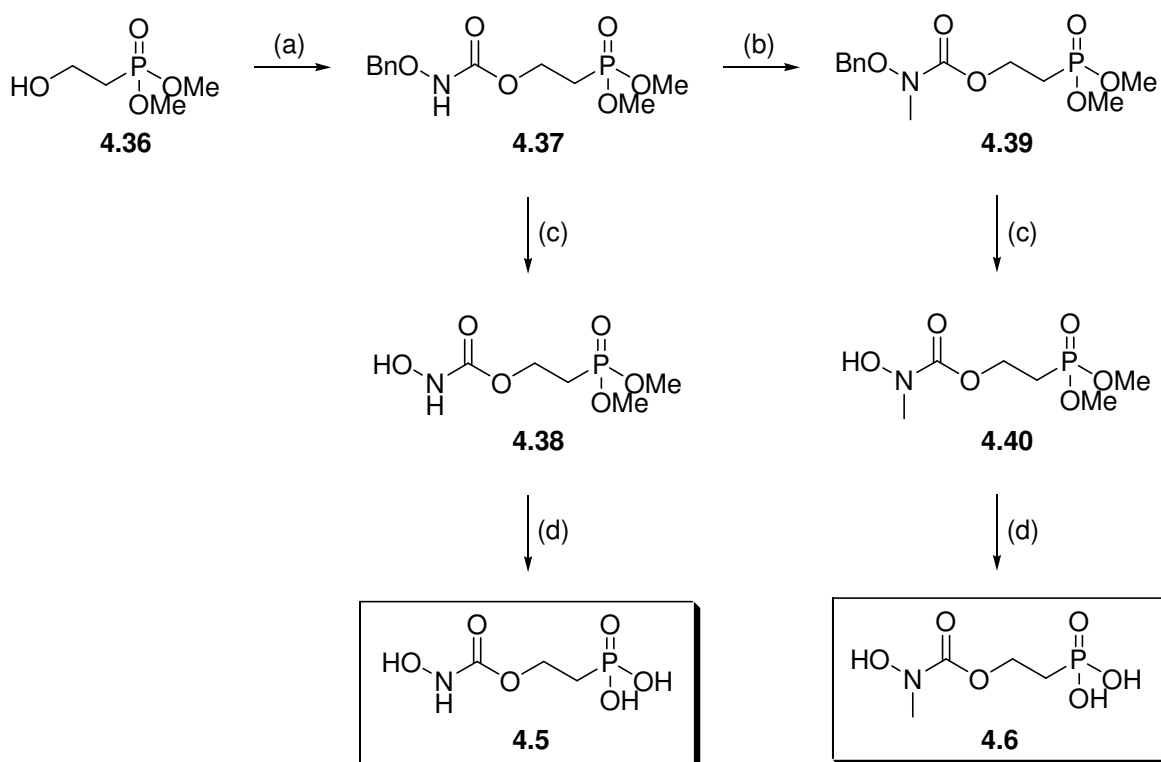
Treatment of carboxylic acid **4.31** with CDI and  $\text{NH}_2\text{-O-Bn}$  afforded the *N*-benzyloxyamide **4.32**. A possible simplification consists of the direct conversion of the ester **4.30** to the *N*-benzyloxyamide **4.32** according to a recently published procedure.<sup>254</sup> A methyl group was introduced by deprotonation of **4.32** with sodium hydride in THF followed by addition of methyl iodide to give **4.34**. Finally, hydroxamic acid **4.3** and its *N*-methylated analogue **4.4** were obtained after catalytic debenzoylation (Pd/C) and deprotection of the phosphonate moieties with two equivalents of TMSBr in dichloromethane.

#### 2.3.3.2.3 Synthesis of compounds **4.5** and **4.6**

The synthesis of the *N*-hydroxy carbamates **4.5** and **4.6** is depicted in Scheme 4.5. Treatment of dimethylhydroxyethyl phosphonate with CDI and *O*-benzylhydroxylamine afforded **4.37**. *N*-methylation, as described for **4.34**, yielded **4.39**. The benzyl protecting groups of **4.37** and **4.39** were removed by catalytic hydrogenolysis (Pd/C) to give **4.38** and **4.40**. Finally, the phosphonate esters were deprotected as outlined above.

<sup>253</sup> (a) Tamaki, K., Ogita, T., Tanzawa, K., Sugimura, Y. *Tetrahedron Lett.* **1993**, 34, 683-686. (b) Altenburger, J. M., Mioskowski, C., d'Orchymont, H., Schirlin D., Schalk, C., Tarnus, C. *Tetrahedron Lett.* **1992**, 33, 5055. (c) Staszak, M. A., Doecke, C. W. *Tetrahedron Lett.* **1994**, 33, 6021-6024. (d) Ando, W., Tsumaki, H. *Synth. Commun.* **1983**, 13, 1053-1056.

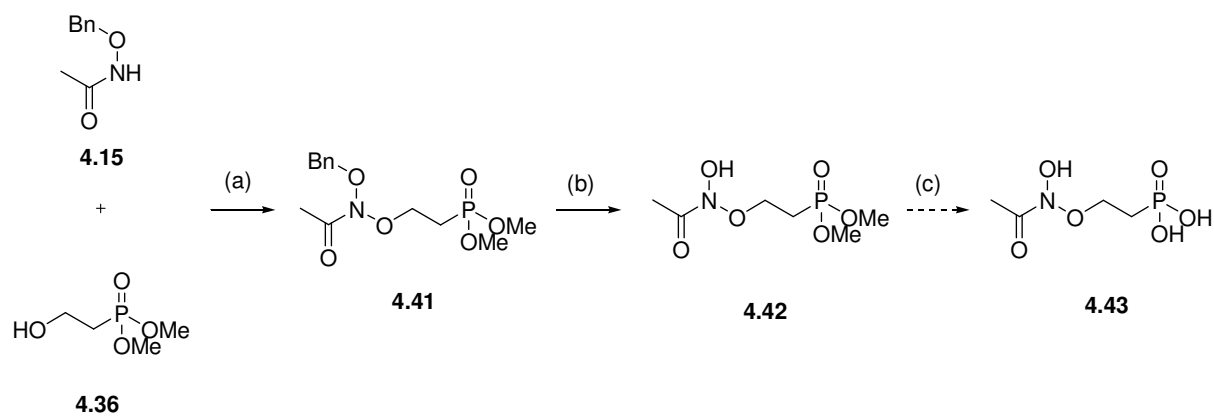
<sup>254</sup> Gissot, A., Volonterio, A., Zanda, M. *J. Org. Chem.* **2005**, 70, 6925-6928.



Scheme 4.5: Reagents and conditions: (a) carbonyldiimidazole,  $\text{NH}_2\text{-O-Bn}\cdot\text{HCl}$ ,  $\text{Et}_3\text{N}$ , rt, 3 h (73%), (b) MeI, NaH, rt, overnight (68%), (c)  $\text{H}_2$ , Pd/C, MeOH, rt, 3 h, (98% and 97%), (d) TMSBr,  $\text{CH}_2\text{Cl}_2$ , rt, 2 h, quantitative.

Analogue **4.43** represents a  $\gamma$ -oxa isoster of FR900098 (Scheme 4.6). In a first attempt, **4.15** was converted to the *N*-chlorohydroxamate in the presence of *t*-butyl hypochlorite.<sup>255</sup> The coupling with **4.36** failed. However, a reaction with bis(trifluoroacetoxy)iodyl benzene (PIFA) yielded compound **4.41**, albeit in moderate yield. Unfortunately, the hydrogenolysis in the next step resulted in a mixture of **4.36** and **4.42** and the latter one was formed in low yield (14% as revealed by NMR). Due to the low yields the synthesis of this compound was abandoned. Analogue **4.43** is probably not very stable, because of a hemiacetal structure.

<sup>255</sup> Glover, S. A., Mo, G. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1728-1739.



Scheme 4.6: Reagents and conditions: (a) PIFA, CH<sub>2</sub>Cl<sub>2</sub>, -30 °C, (b) H<sub>2</sub>, Pd/C, MeOH, rt.



## 2.4. SYNTHESIS OF TRANSITION STATE ANALOGUE

### 2.4.1. INTRODUCTION

Another part of our research project focused on the synthesis of a transition state analogue (**5.1**) of the DOXP reductoisomerase reaction (Figure 5.1). Some studies were performed to unravel the mechanism of DXR. According to Rohmer *et al.* a retroaldolization/aldolization mechanism is more plausible than an  $\alpha$ -ketol rearrangement (*vide supra*). It appears, however, that both the C3 and C4 hydroxyls are important for the catalysis via chelation with a divalent metal ion.<sup>191,216,217</sup> Proteau and coworkers demonstrated that the C3 hydroxyl group is not critical for the binding of DOXP to DXR, but is essential to be converted by the enzyme. Likewise the hydroxyl group at the C4 position of DOXP is important for the isomerisation step, but appears not critical for the binding.<sup>220</sup> Indeed, DOXP analogues lacking the hydroxyl group at C3 or C4 were found to be competitive or reversible mixed type inhibitors of DXR.<sup>191,220</sup> Isosteric analogues of DOXP and MEP with a C–P bond (**1.106** and **1.107** see 1.3.1.4.) have been described by the group of Rohmer.<sup>218,219</sup> While these analogues showed no inhibition for DXR, **1.106** was accepted as an alternative substrate with reduced affinity for DXR. It was argued that turnover of **1.106** is still possible because it differs from the natural substrate at a position that is distant from the part of the molecule undergoing transformation. Although the bridging oxygen of the phosphate group may be implied in interactions with amino acid(s) of the substrate binding site,<sup>218</sup> a C–P bond is crucial to confer resistance to hydrolytic cleavage compared to a P–O bond. Nevertheless, Proteau and coworkers recently reported the phosphate analogues of fosmidomycin and FR900098 to exhibit somewhat lower  $K_i$  values than fosmidomycin when tested on *Synechocystis* DXR (19 and 2 nM versus 58 nM). Because of the presence of a C–P bond in fosmidomycin, however, we decided to retain a phosphonate moiety in the envisaged transition state analogue.

In the transition state the methyl group at the C2 position is essential for affinity of the aldehyde intermediate to the enzyme.<sup>191</sup> The hydroxyl group on the C2 position of the intermediate is also necessary to ligate the divalent cation in the enzyme.<sup>197</sup> Based on this information we decided to synthesize analogue **5.1**, which

possesses all these critical features but has the opposite configuration at C3 (corresponding to C2 of the intermediate) and with an acetyl function in stead of the aldehyde. The acetyl group was chosen since FR900098 shows better affinity for DXR than fosmidomycin. Hence, structural elements believed to stabilize the interaction of the transition state with the enzyme were conserved in **5.1**.

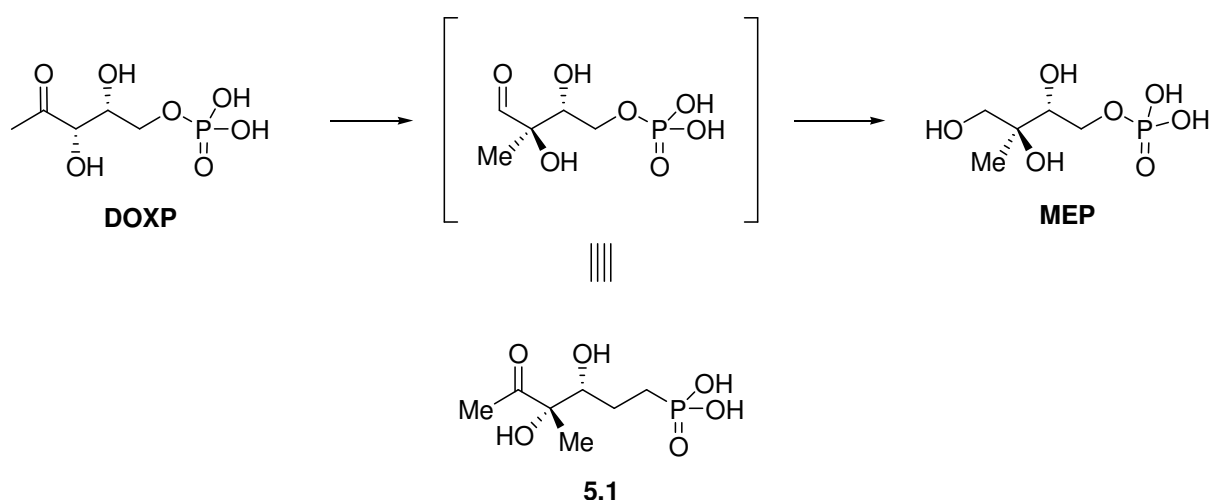
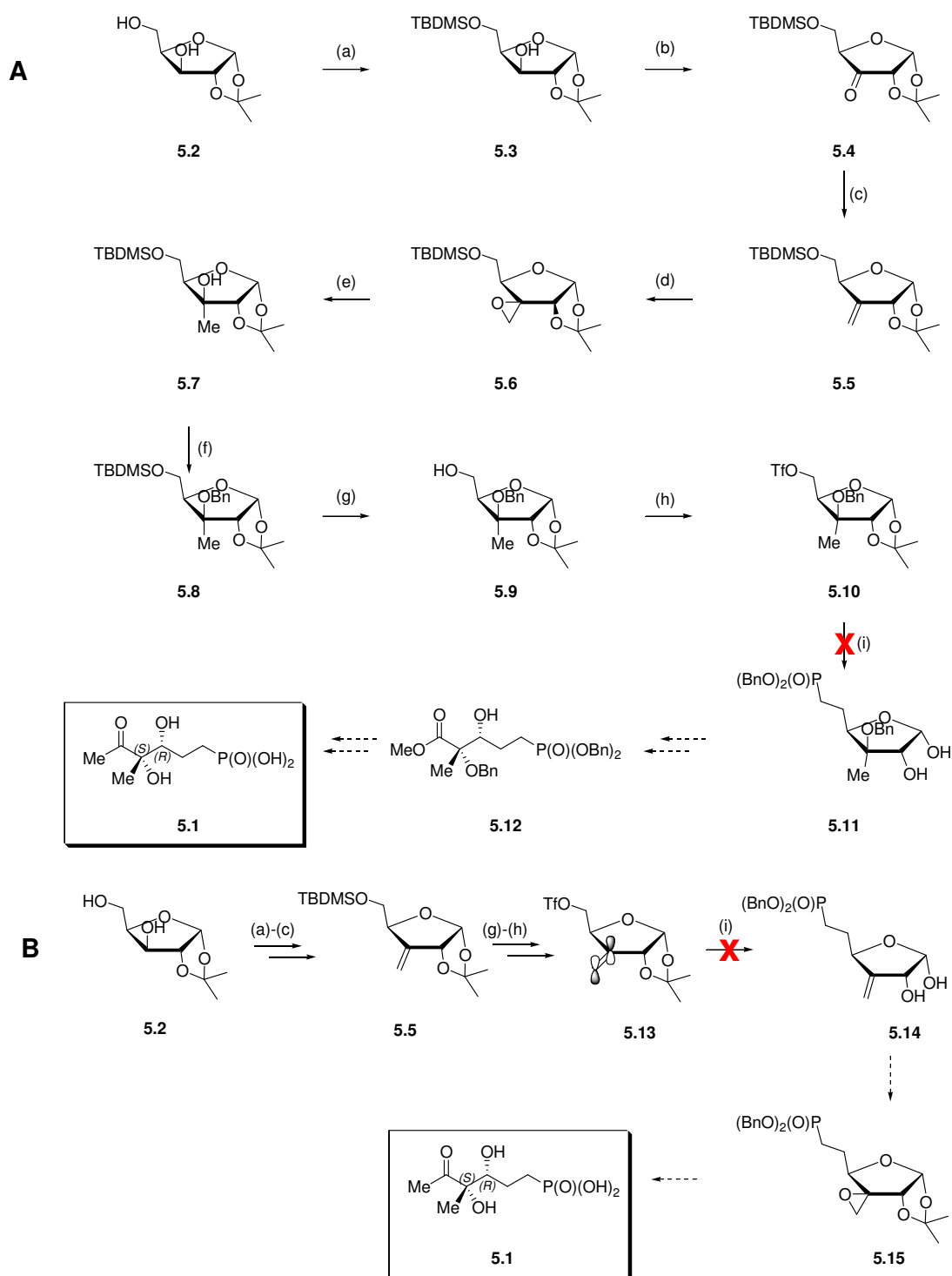


Figure 5.1: Comparison between the transition state and compound **5.1**.

## 2.4.2. SYNTHESIS

Several routes towards the synthesis of transition state analogue **5.1** were explored. Our first attempts started from 1,2-*O*-isopropylidene- $\alpha$ -D-xylofuranose. The xylofuranose moiety can indeed be used as starting material as it contains the same optical configuration as our target compound. The primary OH group of **5.2** was selectively protected as *tert*-butyldimethylsilyl ether **5.3** (Route **A**, scheme 5.1). Swern oxidation yielded ketone **5.4**, which was subjected to a Wittig reaction to afford alkene **5.5**. Compound **5.5** was treated with *m*-chloroperoxybenzoic acid (MCPBA) to yield the desired epoxide **5.6**. The epoxide was reduced with LiAlH<sub>4</sub> to afford the alcohol, whose required configuration was confirmed with NOESY. A contact between C(3)OH and C(2)H was observed. The alcohol at the C3 position was protected with benzyl bromide. Deprotection of the TBDMS protecting group and replacement by a triflate resulted in compound **5.10**.



Scheme 5.1: Attempts to synthesize **5.1** from xylofuranose. Reagents and conditions (a) TBDMSCl, DMAP, pyridine, 0 °C, 1.5 h, (b) (COCl)<sub>2</sub>, DMSO, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, -55 °C→rt, (c) methyltriphenylphosphonium bromide, NaH, DMSO, 3 h, (d) MCPBA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 30 h, (e) LiAlH<sub>4</sub>, THF, 0 °C, 2 h, (f) benzylbromide, NaH, *n*Bu<sub>4</sub>NI, THF, rt, 12 h, (g) TBAF, THF, rt, 4h, (h) trifluoromethanesulfonic acid anhydride, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, -30 °C, 0.5 h, (i) dibenzyl methylphosphonate, HMPA, *n*BuLi, THF, -78 °C.

In the next step the introduction of the phosphonate via an alkylation reaction was envisaged. Despite the resulting complication of the NMR spectra, dibenzyl protection of the phosphonate was chosen, because they could be simultaneously removed with the C3 benzyl protected OH group. Unfortunately, we were unable to obtain compound **5.11**. A literature search revealed that others faced similar problems to introduce the phosphonate group on a hindered  $\beta$ -face of a 1,2-*O*-isopropylidene- $\alpha$ -D-xylofuranose derivative.<sup>219</sup> An attempt to introduce the phosphonate group at the alkene stage was also unsuccessful, most probably due to steric hindrance (Route **B**, scheme 5.1). Therefore this strategy was abandoned and we decided to introduce the envisaged stereochemistry using catalytic asymmetric dihydroxylation as a key step in the synthesis.

This approach started with the introduction of the crucial vicinal diol by using a Sharpless asymmetric hydroxylation on alkene **5.18** (Scheme 5.2). This  $\alpha,\beta$ -unsaturated ester could be obtained by oxidation of 3-(benzyloxy)propan-1-ol (**5.16**) with Dess-Martin periodinane followed by Wittig olefination with the corresponding triphenylphosphorane. This olefination occurred with high (*E*)-selectivity.<sup>256,257</sup> We found that Dess-Martin periodinane gave cleaner results than Swern oxidation. The vicinal diol **5.19** was obtained via Sharpless asymmetric hydroxylation of **5.18** with AD-mix- $\beta$ . The stereoselectivity of the hydroxylation was satisfactory with an enantiomeric excess of 92% (Determined by HPLC analysis on a chiralcel OD-H 250 $\times$ 4.6 column with hexane:EtOH 98:2. To determine this enantiomeric excess we required the enantiomer of **5.19**, which was obtained using AD-mix- $\alpha$ )(Figure 5.2). The optical rotations of compounds **5.19** and **5.20** were 6.4 and 36.0, respectively. Subsequently, the vicinal diol **5.19** was protected as an acetonide to provide **5.20**. Hydrolysis with KOH in aqueous MeOH and acidification provided carboxylic acid **5.21**. This acid was converted to ketone **5.22** upon treatment with 2 equivalents of MeLi. Catalytic hydrogenolysis of **5.22** with Pd/C gave alcohol **5.23**. To introduce the phosphonate group alcohol **5.23** was first converted to iodide **5.24**. This conversion was achieved by employing the chlorodiphenylphosphane/imidazole/iodine reagent combination in toluene. This method allows easy purification by removal of diphenylphosphonic acid by aqueous extraction. Because of the presumed lability,

<sup>256</sup> Kodama, M., Shiobara, Y., Sumitomo, H., Fukuzumi, K., Minami, H., Miyamoto, Y. *J. Org. Chem.* **1988**, 53, 1437-1441.

<sup>257</sup> Chakraborty, T. K., Tapadar, S. *Tetrahedron Lett.* **2003**, 44, 2541-2543.

iodide **5.24** was directly subjected to an Arbusov reaction to furnish the phosphonic ester **5.25**. Finally, the acetonide and the diethyl phosphonic ester were simultaneously deprotected with bromotrimethylsilane to obtain the desired product **5.1** in almost quantitative yield. The final products were purified by RP-HPLC (phenomenex C18 column).

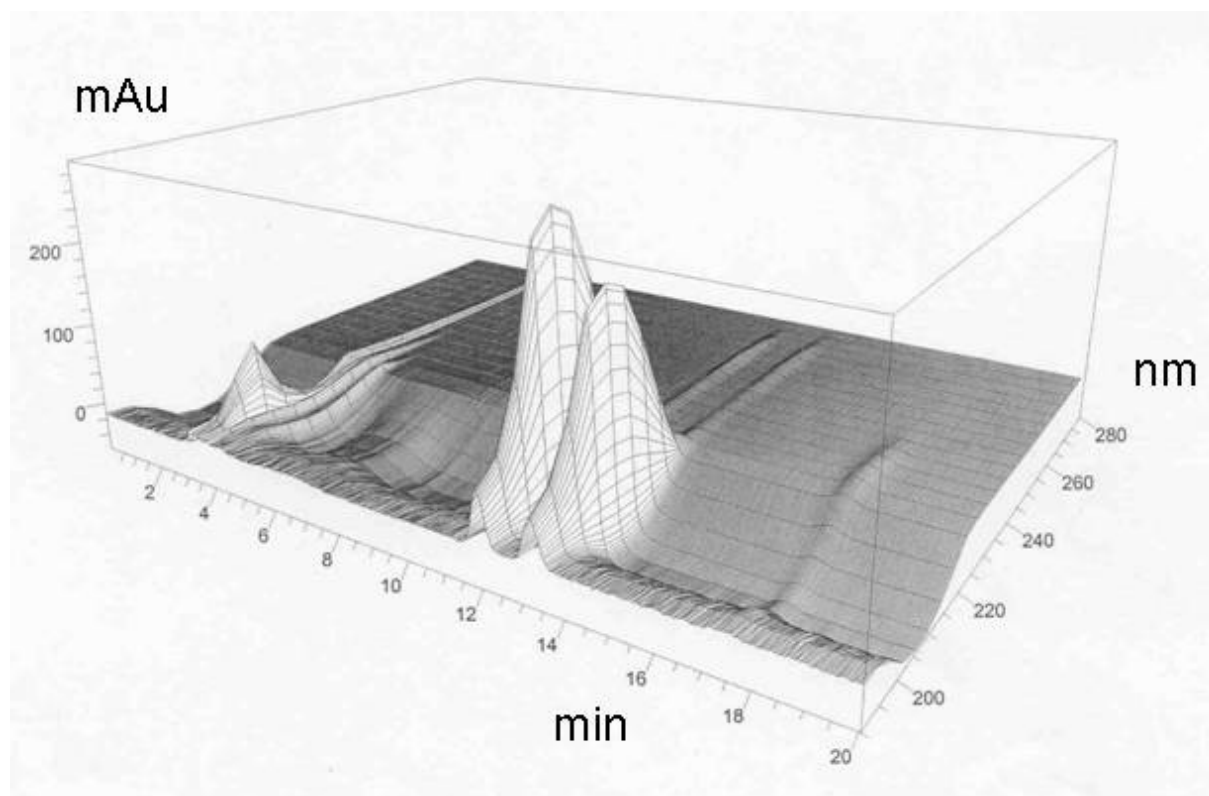
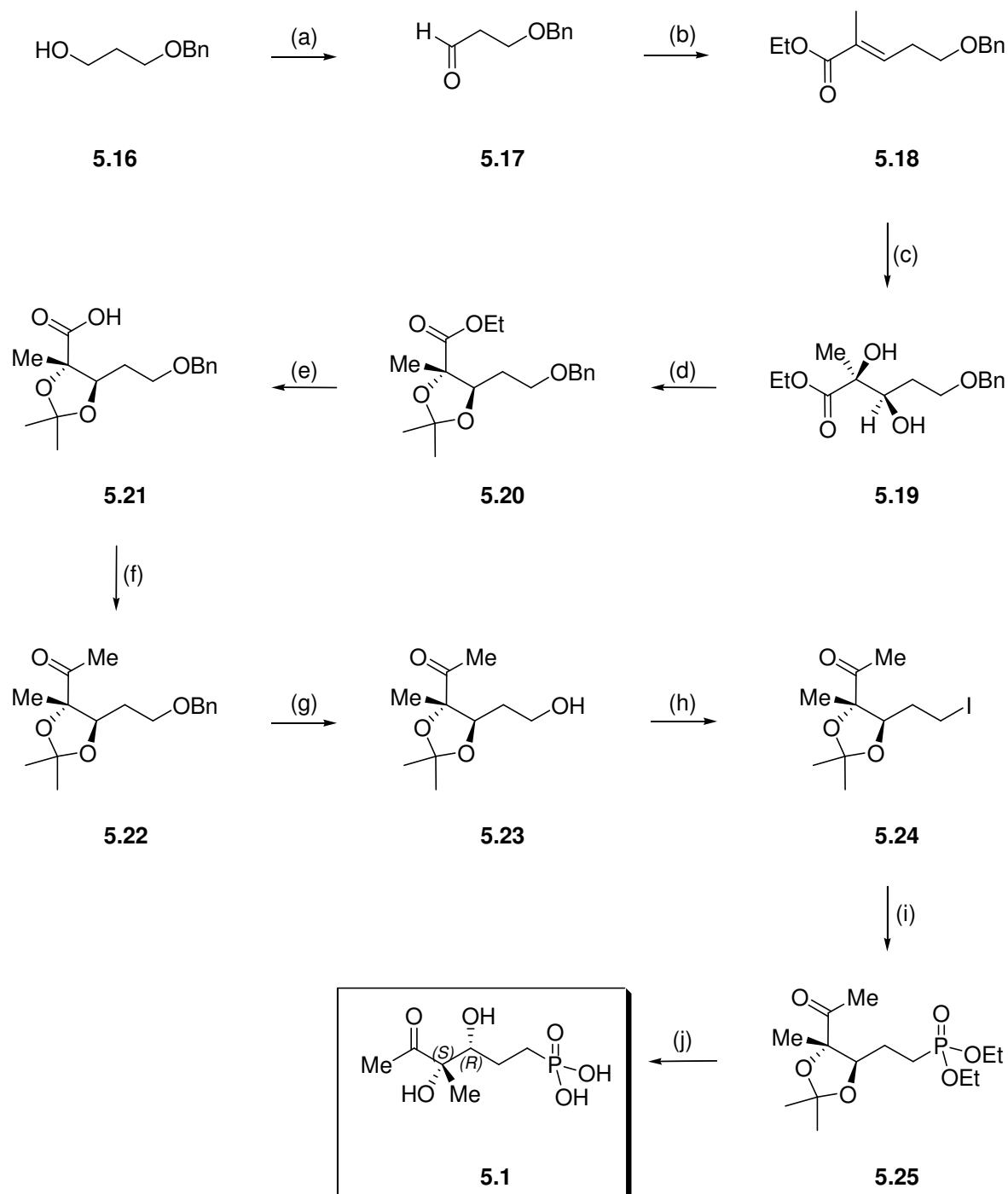


Figure 5.2: Chromatogram of compound **5.19** mixed with its enantiomer on a chiralcel OD-H 250×4.6 column eluted with hexane:EtOH 98:2 obtained by simultaneous UV and DA detection. Ranges: 0.0 to 20.0 min, 190.0 to 280.1 nm, -58.7 to 294.7 mAu.

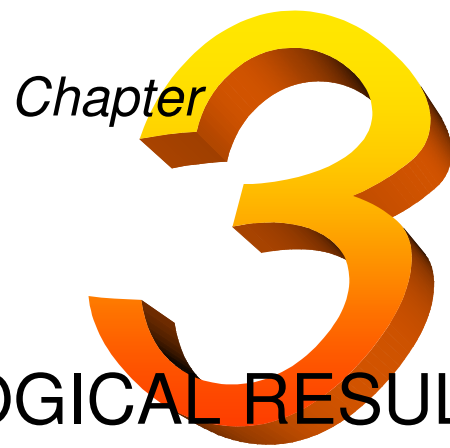


Scheme 5.2: Reagents and conditions (a) Dess-Martin periodinane 15 wt%, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 1 h, (b) Ph<sub>3</sub>P=C(Me)CO<sub>2</sub>Et, toluene, reflux, 30 min (68% over 2 steps), (c) AD-mix β, MeSO<sub>2</sub>NH<sub>2</sub>, *t*BuOH, H<sub>2</sub>O, 0 °C, 24 h (66%, *ee* = 92%), (d) 2,2-dimethoxypropane, TsOH, dry acetone, rt, 4 h (94%), (e) KOH, MeOH, reflux, HCl, 1 h (93%), (f) MeLi, dry THF, 0 °C, 1 h (69%), (g) H<sub>2</sub>, Pd/C, MeOH, rt, 24 h (71%), (h) chlorodiphenylphosphane, imidazole, iodine, NaOH, toluene, rt, 5 min, (i) P(OEt)<sub>3</sub>, reflux, 12 h (81% over 2 steps), (j) TMSBr, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h, quantitative.









*Chapter*

# BIOLOGICAL RESULTS



### 3. BIOLOGICAL RESULTS

All final compounds were tested for inhibition of recombinant *E. coli* DXR. *E. coli* DXR is easier to handle than *P. falciparum* DXR and both show a high degree of similarity. The assay used was based on the photometric measurement of the NADPH turnover associated with the DXR catalyzed reaction. In addition, the *in vitro* antimalarial activity was determined of compounds which scored well in the *E. coli* DXR inhibition assay. Intra-erythrocytic stages of the *P. falciparum* strains D2d or 3D7 were incubated with serial dilutions of the drugs and the viability of the parasites was assessed by their ability to incorporate [<sup>3</sup>H]hypoxanthine into DNA. The exact protocols are described in the experimental part. Fosmidomycin and FR900098 were included as reference compounds. The D2d strain is a progeny of the Indochina clone W2, which is a representative of the most highly resistant strains of *P. falciparum*. It is resistant to chloroquine, quinine, pyrimethamine, cycloguanil and sulfadoxine. Clone 3D7 is resistant to sulfadoxine due to its ability to use exogenous folate, but is sensitive to chloroquine, cycloguanil or pyrimethamine.<sup>7</sup>

#### 3.1. BIOLOGICAL EVALUATION OF COMPOUNDS WITH A MODIFIED RETROHYDROXAMIC ACID

Compared with fosmidomycin, amides **2.1-2.5** showed no interesting inhibitory activity of DXR (Table 6.1). The replacement of the N–OH by a N–Me is not favourable and demonstrates the importance of the N–OH group for metal chelation. Proteau *et al.* confirmed these unpublished results in a recent publication. It is clear that removal of the *N*-hydroxy group would be expected to greatly decrease the potency of the analogues. With the absence of a group capable of chelating the metal ion, the inhibition constants increase to millimolar concentrations. The results for compounds **2.1-2.5** are also in accordance with the results of Walker and Poulter.<sup>221</sup> Thus, a negatively charged or neutral donor atom is required for the chelation of the active site divalent cation. Comparison between the activities of FR900098 with a methyl group in  $\alpha$ -position (**1.130**) and **2.5** demonstrates also the importance of the N–OH group.

Analogues **2.6-2.9**, with an extended N–O bond showed no inhibition either, probably because these larger structures can not be accommodated in the DOXP binding site. The marginal effect of **2.9** ( $IC_{50} = 17 \mu M$ ) needs to be interpreted carefully since this compound suffers from poor solubility thereby possibly disturbing the inhibition assay. Analogue **2.38** showed no inhibition on the recombinant *E. coli* DXR.

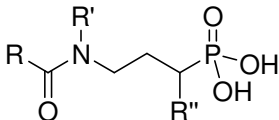
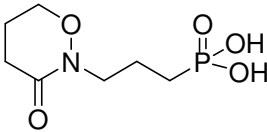
				
compound	R	R'	R''	$IC_{50}$ ( $\mu M$ ) <i>E. coli</i> DXR
<b>fosmidomycin (1.83)</b>	H	OH	H	0.043
<b>FR900098 (1.84)</b>	CH <sub>3</sub>	OH	H	0.043
<b><math>\alpha</math>-Me FR900098 (1.130)*</b>	CH <sub>3</sub>	OH	CH <sub>3</sub>	0.037*
<b>2.1</b>	CH <sub>3</sub>	CH <sub>3</sub>	H	> 30
<b>2.2</b>	CH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	H	> 30
<b>2.3</b>	H	CH <sub>3</sub>	H	> 30
<b>2.4</b>	H	CH <sub>2</sub> CH <sub>3</sub>	H	> 30
<b>2.5</b>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	> 30
<b>2.6</b>	CH <sub>3</sub>	(CH <sub>2</sub> ) <sub>2</sub> OH	H	> 30
<b>2.7</b>	Ph	(CH <sub>2</sub> ) <sub>2</sub> OH	H	> 30
<b>2.8</b>	1-naphtyl	(CH <sub>2</sub> ) <sub>2</sub> OH	H	> 30
<b>2.9</b>	C <sub>15</sub> H <sub>31</sub>	(CH <sub>2</sub> ) <sub>2</sub> OH	H	17
 <b>2.38</b>				> 30

Table 6.1:  $IC_{50}$  values against recombinant *E. coli* DXR (\* unpublished result).

### 3.2. BIOLOGICAL EVALUATION OF COMPOUNDS WITH AN $\alpha$ -ARYL SUBSTITUTED PROPYL SPACER

Compared with fosmidomycin, all  $\alpha$ -aryl substituted analogues (paragraph 2.3.1) were weaker inhibitors of *E. coli* DXR (Table 6.2). Analysis of the order of DXR inhibitory potency in the *N*-acetyl series (**3.2d**  $\approx$  **3.2e** > **3.2a** > **3.2b** > **3.2c**) indicates that the activity is mainly  $+\sigma$ -controlled (4-Cl  $\approx$  3,4-diCl > H > 4-Me > 4-MeO).

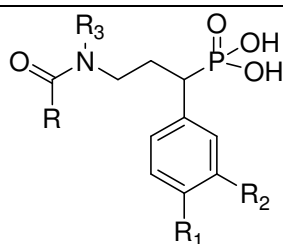
Although generally weaker than fosmidomycin in inhibiting *E. coli* DXR, all  $\alpha$ -substituted analogues studied surpass the activity of fosmidomycin to inhibit the parasite growth. Remarkably, the formyl analogues **3.1c** and **3.1e** consistently outperformed the acetyl derivatives **3.2c** and **3.2e**, both in the enzyme and the parasite growth inhibition assay. An opposite trend was observed for the parent fosmidomycin/FR900098 couple in the parasite growth inhibition assay. This is in accordance with previous studies.<sup>1</sup> Interestingly, a noteworthy correlation was observed between the IC<sub>50</sub> values of **3.1c**, **3.1e** and **3.2a-e** for DXR and their IC<sub>50</sub> for the malaria strains, indicating that *E. coli* DXR inhibition is a useful predictor to estimate the *in vitro* antimalarial activity. Our findings show that this correlation only appears to hold when considering a set of closely related analogues. Indeed, while fosmidomycin is clearly superior to the  $\alpha$ -substituted analogues as DXR inhibitor, it is less active in inhibiting *P. falciparum* growth.

The drop in activity observed for compound **3.17a,c** confirms the importance of the *N*-hydroxyl group for biological activity. However it should be noted that the IC<sub>50</sub>'s of amides **3.17a** and **3.17c** for the 3D7 strain are only 2-fold higher than that of fosmidomycin. These results indicate that the  $\alpha$ -phenyl and  $\alpha$ -(*p*-methoxyphenyl) moieties contribute to the observed activities. This finding is also confirmed by the inactivity of 3-acetamidopropylphosphonic acid as DXR inhibitor.<sup>258</sup>

Compound **3.1e** emerged as the most promising analogue of this series. Its *in vitro* antimalarial activity indicated that it is twelve-fold more active than fosmidomycin and also exceeds the activity of FR900098, the most potent analogue known to date. Apparently, the lipophilic and electronegative properties of the 3,4-dichloro-substitution pattern selectively favour the interaction with the *P. falciparum* DXR

<sup>258</sup> Mercklé, L., Andrés-Gómez, A. d., Dick, B., Cox, R. J., Godfrey, C. R. A. *ChemBioChem*, **2005**, 6, 1866-1874.

homologue. Alternatively, the aromatic ring in the  $\alpha$ -position may improve the capacity of this and related compounds to enter the parasite cells.



compound	R <sub>3</sub>	R	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> (μM) <i>E. coli</i> DXR <sup>a</sup>	IC <sub>50</sub> (μM) Dd2	IC <sub>50</sub> (μM) 3D7
<b>3.2a</b>	OH	CH <sub>3</sub>	H	H	0.311 ± 0.120	0.35	0.55
<b>3.2b</b>	OH	CH <sub>3</sub>	Me	H	0.396 ± 0.061	0.22	0.95
<b>3.1c</b>	OH	H	OMe	H	0.156 ± 0.043	0.20	0.36
<b>3.2c</b>	OH	CH <sub>3</sub>	OMe	H	0.459 ± 0.109	0.27	0.85
<b>3.2d</b>	OH	CH <sub>3</sub>	Cl	H	0.099 ± 0.026	0.095	0.35
<b>3.1e</b>	OH	H	Cl	Cl	0.059 ± 0.020	0.028	0.090
<b>3.2e</b>	OH	CH <sub>3</sub>	Cl	Cl	0.119 ± 0.019	0.090	0.25
<b>3.17a</b>	H	H	H	H	2.391 ± 0.545	7.5	2.0
<b>3.17c</b>	H	OMe	H	H	1.373 ± 0.508	11	2.5
<b>fosmidomycin</b>					0.030 ± 0.008	0.36	1.1
<b>FR900098</b>					0.030 ± 0.008	0.18	0.32

<sup>a</sup>Mean values ± standard deviation of 3 to 5 independent measurements

Table 6.2: IC<sub>50</sub> values of  $\alpha$ -aryl-substituted analogues against recombinant *E. coli* DXR and Dd2 and 3D7 *P. falciparum* strains.

Because of these promising results, compound **3.1e** was scaled up and subjected to an initial *in vivo* study. *P. vinckei* and *P. yoelii* infected mice were treated with 100 mg/kg **3.1e** administered by i.p. injection once daily for 2 days. In parallel,

mice were treated with the same dose of fosmidomycin. *P. vinckei* can be considered as the standard malaria test organism for rodents. Remarkably, in *P. vinckei* infected mice there was no effect with **3.1e**, while complete parasite clearance was observed with fosmidomycin. In *P. yoelii* infected mice, neither **3.1e** nor fosmidomycin proved effective. Further study is necessary to confirm and explain these preliminary results.

Very recently, Kurz *et al.* reported the synthesis and antimalarial activity of prodrugs of  $\alpha$ -arylmethyl substituted derivatives of fosmidomycin.<sup>259</sup> This study suggests that it may be worth trying to make prodrugs of compound **3.1e** and investigate their biological activity.

### **3.3. BIOLOGICAL EVALUATION OF CYCLOPENTYL COMPOUNDS**

Amongst the fosmidomycin analogues in which the C–C–C spacer is part of a cyclopentane ring, the *trans* analogues proved notably more active than the *cis* isomers but less than fosmidomycin (Table 6.3). This is in agreement with recent results of our group obtained with cyclopropane fosmidomycin analogues, where a *trans* orientation of the phosphonate group and the hydroxyamide moiety also yielded the most potent inhibitor.<sup>213</sup> Remarkably, in the cyclopentane series, the inhibitory activity of the formyl analogues surpassed that of the acetyl derivatives, while the opposite trend was observed in the cyclopropane series.<sup>213</sup> In view of the acceptable activity of *trans*-**3.3** it seems worthwhile to separate the racemate and to test the pure enantiomers on their biological activity. Moreover, regarding the acceptable activities of the conformationally restricted analogues *trans*-**3.3** and **1.95** it would also be interesting to synthesize less rigid compounds, e.g.  $\beta$ - or  $\gamma$ -methylated compounds.

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<sup>259</sup> Schlüter, K., Walter, R. D., Bergmann, B., Kurz, T. *Eur. J. Med. Chem.* **2006**, 41, 1385-1397.

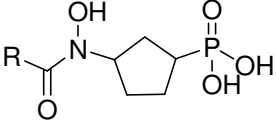
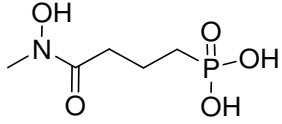
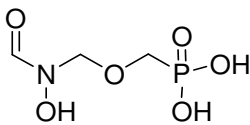
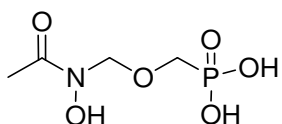
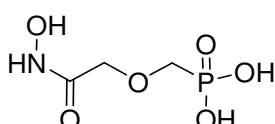
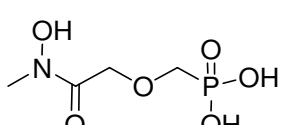
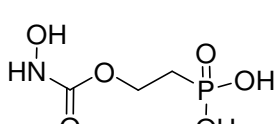
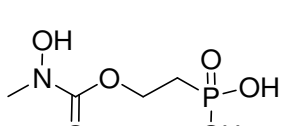
		
Compounds	R	IC <sub>50</sub> (μM)
fosmidomycin		0.029
FR900098		0.035
<i>trans</i> - <b>3.3</b>	H	0.20
<i>cis</i> - <b>3.3</b>	H	2.3
<i>trans</i> - <b>3.4</b>	CH <sub>3</sub>	2.3
<i>cis</i> - <b>3.4</b>	CH <sub>3</sub>	12

Table 6.3: Inhibitory activity of cyclopentyl analogues on recombinant *E. coli* DXR enzyme.

### 3.4. BIOLOGICAL EVALUATION OF OXA COMPOUNDS

The biological results for the oxa analogues are summarized in table 6.4. In the series with the inversed hydroxamates and carbamates the *N*-methyl substituted analogues proved superior to their non-methylated analogues. Compound **1.71**, originally made by Rohmer *et al.*, was marginally less active than fosmidomycin and FR900098 in the *E. coli* DXR inhibition assay. The results of **4.4** and **4.6** show that introduction of an oxygen atom in the propyl chain is more favourable in β-position of the phosphonate moiety than in γ-position. Introduction of an oxygen atom in the γ-position of the phosphonate moiety generates carbamates **4.5** and **4.6** which were weak inhibitors of *E. coli* DXR, as shown by the relatively high IC<sub>50</sub> values. Apparently the interaction observed between the C4 hydroxyl group of the substrate with Glu152, Asn227 and Lys228 cannot be accommodated by the γ-oxygen. In contrast, compound **4.4** proved to be almost equipotent to FR900098 and marginally more active than compound **1.71**. Comparison of IC<sub>50</sub> values of analogues **4.2** and **4.4** demonstrates that, when combined with a β-oxa modification, the hydroxamate seems slightly favourable to the *N*-hydroxyacetamido group as metal chelating moiety.



compound	structure	IC <sub>50</sub> (μM) <i>E. coli</i> DXR <sup>a</sup>	IC <sub>50</sub> (μM) 3D7
1.71		0.093	0.994
4.1		1.105	5.192
4.2		0.087	0.348
4.3		>3	-
4.4		0.072	0.228
4.5		>3	-
4.6		4.942	-
<b>fosmidomycin</b>		0.054	0.983
<b>FR900098</b>		0.062	0.373

<sup>a</sup>Mean values of 3 to 5 independent measurements

Table 6.4: Inhibitory activity of oxa analogues on recombinant *E. coli* DXR enzyme.

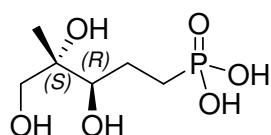
The introduction of an oxygen atom in the linker does not contribute favourably to the hydrophobic interactions which are present in the crystal structure of DXR from Sweeney *et al.*, although on this *E. coli* DXR inhibition assay analogue **4.4** was more

potent than analogue **1.71** with the methylene linker. This points out that an oxygen atom in  $\beta$ -position slightly influences the ionisation and as such the binding, even if hydrophobic interactions are playing a part. Likewise, it is also possible that the ether linkage is still hydrophobic enough to ensure a tight bonding of the inhibitor. In order to confirm that there is no hydrogen bonding involved, a crystal structure of DXR complexed with analogue **4.4** is highly desirable.

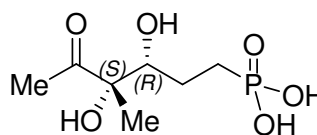
Furthermore, compound **4.2** outperformed its formyl analogue **4.1**. An opposite trend was observed in the fosmidomycin/FR900098 couple, where fosmidomycin is superior to FR900098. Nevertheless, in this series of compounds fosmidomycin stays the best inhibitor on this *E. coli* DXR inhibition assay.

On the *P. falciparum* 3D7 strain, compounds **4.2** and **4.4** surpassed fosmidomycin and even FR900098, with analogue **4.4** as the most promising analogue. Analogue **4.4** is almost twice as active as FR900098 and four times more active than fosmidomycin.

### 3.5. BIOLOGICAL EVALUATION OF TRANSITION STATE ANALOGUE 5.1



**1.107**



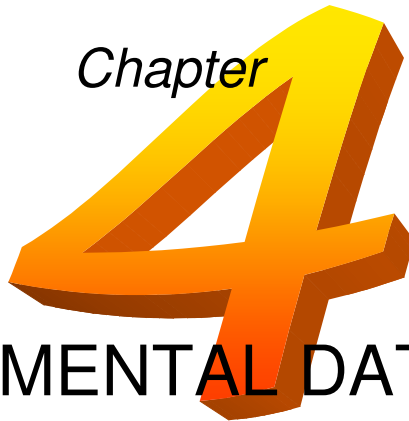
**5.1**

Analogue **1.107** is the only structure reported to date that can be considered as a transition state analogue. It concerns a phosphonate analogue of MEP. The influence of **1.107** on the growth of *E. coli* was tested by the agar diffusion method on agar plates (5 cm diameter) inoculated with a bacteria suspension (100  $\mu$ L,  $5 \times 10^7$  cells). No growth inhibition zone was observed around 6 mm Whatman No. 1 paper disks in the presence of **1.107** (50 or 100  $\mu$ g), whereas the presence of fosmidomycin (10  $\mu$ g), induced the formation of a clear growth inhibition zone. Also the activity on *E. coli* DXR was investigated. DXR was capable of converting **1.107** into **1.106**, which is

a phosphonate analogue of DOXP. Unfortunately, an intermediate analogue **5.1** did not demonstrate remarkable inhibition on *E. coli* DXR either.



*Chapter*



# EXPERIMENTAL DATA



## **4. EXPERIMENTAL DATA**

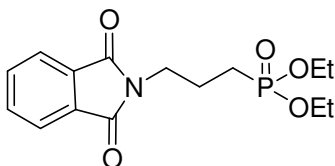
### **4.1. SYNTHESIS**

#### **4.1.1. GENERAL**

IUPAC names were generated with Chemdraw Ultra 8.0 (Chemoffice 2004, Cambridge Soft, Cambridge, USA). All reactions were carried out under inert (N<sub>2</sub>) atmosphere. Precoated Merck silica gel F<sub>254</sub> plates and precoated Macherey-Nagel (Düren, Germany) silica gel F<sub>254</sub> plates were used for TLC and spots were examined under UV light at 254 nm and revealed by a phosphomolybdic-cerium sulphate solution, iodine vapour or bromocresol green solution. Column chromatography was performed on ICN silica gel (63-200 µm). NMR spectra were obtained with a Varian Mercury 300 spectrometer. Chemical shifts are given in parts per million (ppm) ( $\delta$  relative to residual solvent peak, in the case of CDCl<sub>3</sub> 7.26 ppm for <sup>1</sup>H and 77.4 ppm for <sup>13</sup>C. Coupling constants are expressed in Hz. Abbreviations used are: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. s = broad signal. All signals assigned to hydroxyl and to amino groups were exchangeable with D<sub>2</sub>O. Structural assignment was confirmed with COSY, NOEDIF, NOESY, DEPT and/or HMQC if necessary. Mass spectra and exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof 2, Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a ACN/water (1:1) mixture at 3µL/min. Most chemicals were obtained from Sigma-Aldrich or Acros Organics and were used without further purification.

#### 4.1.2. RECIPES AND SPECTRAL DATA

##### Diethyl [3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)propyl]phosphonate (**2.11**).

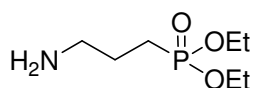


*N*-(3-Bromopropyl)phthalimide (3 g, 11.2 mmol) was added to triethyl phosphite (9.3 g, 60.0 mmol) slowly at room temperature. The reaction mixture was refluxed for 12 h, and then excess triethyl phosphite and other volatile compounds were distilled out under reduced pressure (3 mmHg) at 60 °C. The pure phosphonate **2.11** (3.6 g, 98%) was obtained as a pale yellow viscous oil. The <sup>1</sup>H NMR spectrum matched the published spectrum.<sup>260</sup>

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.08 (t, *J* = 7.0 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.59 – 1.51 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 1.77 – 1.62 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 3.51 (t, *J* = 6.7 Hz, 2H, NCH<sub>2</sub>), 3.85 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 7.51 (m, 2H, arom. H), 7.59 (m, 2H, arom. H) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>15</sub>H<sub>21</sub>NO<sub>5</sub>P [M+H]<sup>+</sup>: 326.1157, found: 326.1160.

##### Diethyl 3-aminopropylphosphonate (**2.12**)



Hydrazine monohydrate (5.2 g, 10.4 mmol) was added dropwise to a solution of phthalimide **2.11** (1.12 g, 3.5 mmol) in ethanol (50 mL) at room temperature. The reaction mixture was heated under reflux overnight. The precipitated phthalyl hydrazide was filtered, and the solvent was removed under reduced pressure. Evaporation of the solvent produced phosphonate **2.12** as a yellow viscous oil. The crude phosphonate **2.12** was purified by flash chromatography to yield pure **2.12** (1.9 g, 94%) (CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia in MeOH, 75:24:1). The spectral data matched with the literature data.<sup>261</sup>

<sup>260</sup> Chun, Y.-J., Park, J.-H., Oh, G.-M., Hong, S.-I., Kim, Y.-J. *Synthesis* **1994**, 909-910.

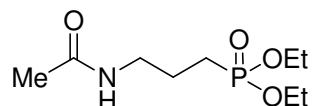
<sup>261</sup> Bako, P., Novak, T., Ludanyi, K., Pete, B., Toke, L., Keglevich, G. *Tetrahedron: Asymmetry* **1999**, 10, 2373-2380.



**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.33 (t, *J* = 7.0 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.42 (bs, 2H, NH<sub>2</sub>), 1.85 – 1.79 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 2.86 (t, *J* = 6.0 Hz, 2H, NCH<sub>2</sub>), 4.15 – 4.06 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>7</sub>H<sub>19</sub>NO<sub>3</sub>P [M+H]<sup>+</sup>: 196.1103, found: 196.0752.

### Diethyl 3-acetamidopropylphosphonate (**2.13**)

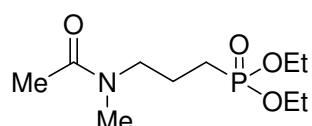


To a solution of phosphonate **2.12** (1.47 g, 7.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) was added acetylchloride (1.18 g, 7.5 mmol) and pyridine (1.19 g, 7.5 mmol). The reaction was cooled by 0 °C. After 3 h the reaction was finished as revealed by TLC and the reaction mixture was evaporated and purified by flash chromatography to yield **2.13** (1.46 g, 82%)(CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 9:1).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.33 (t, *J* = 7.0 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.85 – 1.79 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 1.81 (s, 3H, CH<sub>3</sub>C=O), 3.05 (m, 2H, NCH<sub>2</sub>), 4.15 – 4.06 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 7.81 (br. s, 1H, NH) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>9</sub>H<sub>21</sub>NO<sub>4</sub>P [M+H]<sup>+</sup>: 238.1209, found: 238.1231.

### Diethyl [3-(*N*-methylacetamido)propyl]phosphonate (**2.14**)



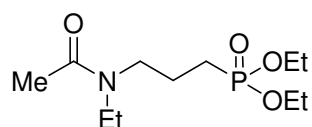
To a solution of phosphonate **2.13** (0.55 g, 2.4 mmol) in THF (9 mL) was added sodium hydride (64.2 mg, 2.6 mmol) in small portions. This reaction mixture was stirred by room temperature for 30 minutes. Iodomethane (0.38 g, 2.6 mmol) was added dropwise to the reaction mixture and the solution was stirred over night. The crude reaction mixture was evaporated *in vacuo* and purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 99:1→98:2→97:3) to yield **2.14** as a viscous oil (0.44 g, 72%).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.33 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.65 – 1.86 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 2.16 and 2.21 (2 × s, 3H, CH<sub>3</sub>C=O, major and minor form), 2.82 – 2.99 (2

× s, 3H, NCH<sub>3</sub>, minor and major form), 3.62 – 3.85 (m, 2H, NCH<sub>2</sub>), 4.10 – 4.31 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>10</sub>H<sub>23</sub>NO<sub>4</sub>P [M+H]<sup>+</sup>: 252.1366, found: 252.1357.

### Diethyl 3-(*N*-ethylacetamido)propylphosphonate (**2.15**)

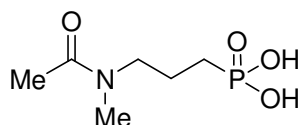


To a solution of phosphonate **2.13** (0.36 g, 1.5 mmol) in THF (10 mL) was added sodium hydride (43.5 mg, 1.8 mmol) in small portions. This reaction mixture was stirred by room temperature for 30 minutes. Iodoethane (0.35 g, 2.3 mmol) was added dropwise to the reaction mixture and the solution was stirred overnight. The crude reaction mixture was evaporated *in vacuo* and purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 99:1→98:2→97:3) to yield **2.15** as a viscous oil (0.27 g, 67%).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.02 – 1.63 (m, 9H, OCH<sub>2</sub>CH<sub>3</sub> and NCH<sub>2</sub>CH<sub>3</sub>), 1.65 – 1.78 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 1.82 and 2.11 (2 × s, 3H, CH<sub>3</sub>C=O, minor and major form), 3.22 – 3.38 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub> and NCH<sub>2</sub>CH<sub>3</sub>), 3.97 – 4.13 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>11</sub>H<sub>25</sub>NO<sub>4</sub>P [M+H]<sup>+</sup>: 266.1521, found: 266.1533.

### [3-(*N*-Acetyl-*N*-methyl-amino)-propyl]-phosphonic acid (**2.1**)



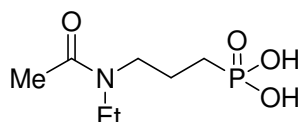
Trimethylsilyl iodide (240 mg, 1.2 mmol) was added dropwise at 0 °C with stirring to a solution of phosphonic ester **2.14** (100 mg, 0.4 mmol) in dichloromethane (4 mL), and stirring was continued at room temperature for 2 h. Organic solvents were removed *in vacuo*. The crude yellow foam was dissolved in ethanol, decolourized over a active carbon patch and subsequently precipitated from ethanol/Et<sub>2</sub>O to yield the pure phosphonic acid **2.1** in almost quantitative yield.

**<sup>1</sup>H-NMR** (300 MHz, D<sub>2</sub>O): mixture of amide rotamers  $\delta$  = 1.42 – 1.78 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 1.85 and 1.93 (2 × s, 3H, CH<sub>3</sub>C=O, major and minor form), 2.74 – 2.92 (2 × s, 3H, NCH<sub>3</sub>, minor and major form), 3.20 – 3.54 (m, 2H, NCH<sub>2</sub>) ppm.

**<sup>13</sup>C-NMR** (75 MHz, D<sub>2</sub>O): mixture of amide rotamers  $\delta$  = 21.04 and 20.44 (CH<sub>3</sub>C=O, major and minor form), 22.06 (d, <sup>2</sup>J<sub>C,P</sub> = 3.2 Hz, CH<sub>2</sub>CH<sub>2</sub>P) and 21.92 (d, <sup>2</sup>J<sub>C,P</sub> = 3.5 Hz, CH<sub>2</sub>CH<sub>2</sub>P), 25.44 (d, J<sub>C,P</sub> = 130.7 Hz, CH<sub>2</sub>CH<sub>2</sub>P) and 25.14 (d, J<sub>C,P</sub> = 131.6 Hz, CH<sub>2</sub>CH<sub>2</sub>P), 33.50 and 36.35 (NCH<sub>3</sub>), 49.15 (d, <sup>3</sup>J<sub>C,P</sub> = 20.2 Hz, NCH<sub>2</sub>) and 52.43 (d, <sup>3</sup>J<sub>C,P</sub> = 19.5 Hz, NCH<sub>2</sub>), 174.1 (CH<sub>3</sub>C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>6</sub>H<sub>13</sub>NO<sub>4</sub>P [M-H]<sup>-</sup>: 194.0581, found: 194.0224.

### [3-(*N*-Acetyl-*N*-ethyl-amino)-propyl]-phosphonic acid (**2.2**)



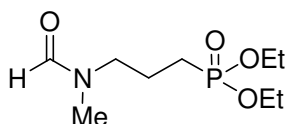
Trimethylsilyl iodide (208 mg, 2.0 mmol) was added dropwise at 0 °C with stirring to a solution of phosphonic ester **2.15** (92 mg, 0.35 mmol) in dichloromethane (4 mL), and stirring was continued at room temperature for 2 h. Organic solvents were removed *in vacuo*. The crude yellow foam was dissolved in ethanol, decolourized over a active carbon patch and subsequently precipitated from ethanol/Et<sub>2</sub>O to yield the pure phosphonic acid **2.2** in almost quantitative yield.

**<sup>1</sup>H-NMR** (300 MHz, D<sub>2</sub>O): mixture of amide rotamers  $\delta$  = 0.91 and 0.99 (2 × t, J = 7.0 Hz, 3H, NCH<sub>2</sub>CH<sub>3</sub>, major and minor form), 1.08 – 1.21 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 1.46 – 1.69 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 1.71 and 1.94 (2 × s, 3H, CH<sub>3</sub>C=O, minor and major form), 3.09 – 3.28 (m, 4H, NCH<sub>2</sub>CH<sub>3</sub> and NCH<sub>2</sub>CH<sub>2</sub>) ppm.

**<sup>13</sup>C-NMR** (75 MHz, D<sub>2</sub>O): mixture of amide rotamers  $\delta$  = 12.06 and 12.86 (NCH<sub>2</sub>CH<sub>3</sub>), 20.49 and 20.78 (CH<sub>3</sub>C=O, major and minor form), 22.49 (d, <sup>2</sup>J<sub>C,P</sub> = 4.8 Hz, CH<sub>2</sub>CH<sub>2</sub>P) and 23.41 (d, <sup>2</sup>J<sub>C,P</sub> = 4.9 Hz, CH<sub>2</sub>CH<sub>2</sub>P), 26.41 (d, J<sub>C,P</sub> = 131.6 Hz, CH<sub>2</sub>CH<sub>2</sub>P) and 26.59 (d, J<sub>C,P</sub> = 131.3 Hz, CH<sub>2</sub>CH<sub>2</sub>P), 41.19 and 43.99 (NCH<sub>2</sub>CH<sub>3</sub>), 46.95 (d, <sup>3</sup>J<sub>C,P</sub> = 20.7 Hz, NCH<sub>2</sub>) and 50.20 (d, <sup>3</sup>J<sub>C,P</sub> = 20.1 Hz, NCH<sub>2</sub>), 173.6 (CH<sub>3</sub>C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>7</sub>H<sub>15</sub>NO<sub>4</sub>P [M-H]<sup>-</sup>: 208.0737, found: 208.0753.

### Diethyl [3-(*N*-methylformamido)propyl]phosphonate (**2.17**)

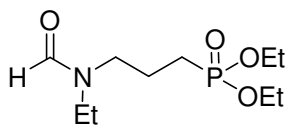


To a solution of *N*-methylformamide (0.3 g, 5 mmol) in DMF (5 mL) was added sodium hydride (144 mg, 6 mmol). The reaction mixture was stirred for 30 minutes and diethyl(3-bromo-propyl)phosphonate (1.35 g, 5 mmol) was added to this solution and stirred for 16 h. All volatile compounds were removed under reduced pressure and the residue was diluted with CH<sub>2</sub>Cl<sub>2</sub> and extracted with water. The organic layer was dried over MgSO<sub>4</sub> and evaporated under reduced pressure. The crude residue was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 98:2→97:3) to yield **2.17** as a viscous oil (0.61 g, 52%).

**<sup>1</sup>H-NMR** (300 MHz, DMSO):  $\delta$  = 1.20 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.60 – 1.64 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 2.69 and 2.86 (2 × s, 3H, NCH<sub>3</sub>, major and minor form), 3.23 – 3.32 (m, 2H, NCH<sub>2</sub>), 7.95 (s, 1H, HC=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>9</sub>H<sub>21</sub>NO<sub>4</sub>P [M+H]<sup>+</sup>: 238.1209, found: 238.1226.

### Diethyl [3-(*N*-ethylformamido)propyl]phosphonate (**2.18**)

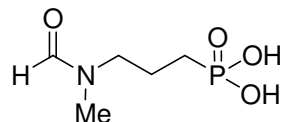


To a solution of *N*-ethylformamide (0.36 g, 5 mmol) in DMF (5 mL) was added sodium hydride (144 mg, 6 mmol). The reaction mixture was stirred for 30 minutes and diethyl(3-bromo-propyl)phosphonate (1.35 g, 5 mmol) was added to this solution and stirred for 16 h. All volatile compounds were removed under reduced pressure and the residue was diluted with CH<sub>2</sub>Cl<sub>2</sub> and extracted with water. The organic layer was dried over MgSO<sub>4</sub> and evaporated under reduced pressure. The crude residue was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 97:3) to yield **2.18** as a viscous oil (0.85 g, 72%).

**<sup>1</sup>H-NMR** (300 MHz, DMSO): mixture of amide rotamers  $\delta$  = 0.99 and 1.06 (2 × t, *J* = 7.2 Hz, 3H, NCH<sub>2</sub>CH<sub>3</sub>, two forms), 1.20 (t, *J* = 7.2 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.62 – 1.63 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 3.12 – 3.29 (m, 4H, NCH<sub>2</sub>CH<sub>3</sub> and NCH<sub>2</sub>CH<sub>2</sub>), 3.93 – 3.98 (m, 4H, POCH<sub>2</sub>CH<sub>3</sub>) 7.95 and 8.01 (2 × s, 1H, HC=O, two forms) ppm.

**Exact mass** (ESI-MS): calculated for  $C_{10}H_{23}NO_4P$   $[M+H]^+$ : 252.1365, found: 252.1372.

**[3-(*N*-Formyl-*N*-methyl-amino)-propyl]-phosphonic acid (2.3)**

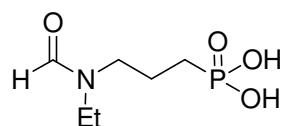


**$^1H$ -NMR** (300 MHz,  $D_2O$ ): mixture of amide rotamers  $\delta$  = 1.36–1.47 (m, 2H,  $CH_2CH_2P$ ), 1.61–1.71 (m, 2H,  $CH_2CH_2P$ ), 2.41 and 2.63 (2  $\times$  s, 3H,  $NCH_3$ ), 3.16–3.27 (m, 2H,  $NCH_2$ ), 7.81 and 7.92 (2  $\times$  s, 1H,  $HC=O$ , minor and major form) ppm.

**$^{13}C$ -NMR** (75 MHz,  $D_2O$ ): mixture of amide rotamers  $\delta$  = 21.44 (d,  $^2J_{CP}$  = 3.0 Hz,  $CH_2CH_2P$ ) and 22.60 (d,  $^2J_{CP}$  = 3.8 Hz,  $CH_2CH_2P$ ), 25.44 (d,  $J_{CP}$  = 134.5 Hz,  $CH_2CH_2P$ ), 32.60 and 35.07 ( $NCH_3$ ), 51.95 (d,  $^3J_{CP}$  = 18.9 Hz,  $NCH_2$ ) and 48.72 (d,  $^3J_{CP}$  = 19.7 Hz,  $NCH_2$ ), 165.3 ( $HC=O$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $C_5H_{11}NO_4P$   $[M-H]^-$ : 180.0424, found: 180.0449.

**[3-(*N*-Ethyl-*N*-formyl -amino)-propyl]-phosphonic acid (2.4)**

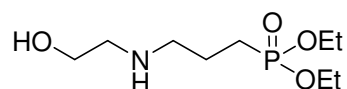


**$^1H$ -NMR** (300 MHz,  $D_2O$ ): mixture of amide rotamers  $\delta$  = 0.93 and 1.00 (2  $\times$  t, 3H,  $NCH_2CH_3$ , major and minor form), 1.10 – 1.19 (m, 2H,  $CH_2CH_2P$ ), 1.46 – 1.71 (m, 2H,  $CH_2CH_2P$ ), 3.11 – 3.28 (m, 4H,  $NCH_2CH_3$  and  $NCH_2CH_2$ ), 7.81 and 7.87 (2  $\times$  s, 1H,  $HC=O$ , major and minor form) ppm.

**$^{13}C$ -NMR** (75 MHz,  $D_2O$ ): mixture of amide rotamers  $\delta$  = 11.96 and 13.80 ( $NCH_2CH_3$ ), 22.17 (d,  $^2J_{C,P}$  = 4.8 Hz, ,  $CH_2CH_2P$ ) and 23.33 (d,  $^2J_{C,P}$  = 4.9 Hz, ,  $CH_2CH_2P$ ), 26.41 (d,  $J_{C,P}$  = 131.2 Hz,  $PCH_2$ ) and 27.26 (d,  $J_{C,P}$  = 131.0 Hz,  $PCH_2$ ), 37.65 and 43.22 ( $NCH_2CH_3$ ), 49.38 (d,  $^3J_{C,P}$  = 19.0 Hz,  $NCH_2$ ), 173.6 ( $HC=O$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $C_6H_{13}NO_4P$   $[M-H]^-$ : 194.0581, found: 194.0591.

**Diethyl [3-(2-hydroxyethylamino)propyl]phosphonate (2.21)**

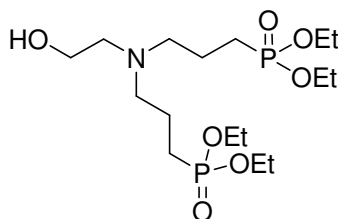


A solution of diethyl 3-bromopropylphosphonate (2.0 mL, 10 mmol) in acetonitrile (5 mL) was added to a suspension of ethanolamine (3.0 mL, 50 mmol) and potassium carbonate (14 g, 0.1 mol) in acetonitrile (15 mL). When TLC revealed that all starting material was gone, excess potassium carbonate was filtered off and the solvent of the filtrate was removed under reduced pressure. The residue was mixed with celite and was subsequently purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3$  in MeOH 85:14:1) yielding **2.21** (1.64 g, 68%) as a yellow oil. Only small traces of the disubstituted ethanolamine were formed (0.02%).

**$^1\text{H}$  NMR** (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 1.20 (t,  $J$  = 7.2 Hz, 6H,  $\text{OCH}_2\text{CH}_3$ ), 1.51 – 1.62 (m, 2H,  $\text{CH}_2\text{CH}_2\text{P}$ ), 1.66 – 1.77 (m, 2H,  $\text{CH}_2\text{CH}_2\text{P}$ ), 2.48 – 2.56 (m, 4H,  $\text{CH}_2\text{NCH}_2\text{CH}_2\text{OH}$ ), 3.38 – 3.43 (m, 2H,  $\text{NCH}_2\text{CH}_2\text{OH}$ ), 3.89 – 4.00 (m, 4H,  $\text{OCH}_2\text{CH}_3$ ), 4.41 (br. s, 1H, OH) ppm.

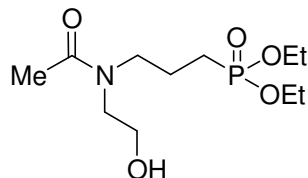
**Exact mass** (ESI-MS): calculated for  $\text{C}_9\text{H}_{21}\text{NO}_4\text{P}$   $[\text{M}-\text{H}]^-$ : 238.1207, found: 238.1236.

#### Side product 2.22



**$^1\text{H}$  NMR** (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 1.20 (t,  $J$  = 7.2 Hz, 12H,  $\text{OCH}_2\text{CH}_3$ ), 1.45 – 1.61 (m, 4H,  $\text{CH}_2\text{CH}_2\text{P}$ ), 1.63 – 1.74 (m, 4H,  $\text{CH}_2\text{CH}_2\text{P}$ ), 2.40 – 2.49 (m, 6H,  $(\text{CH}_2)_2\text{NCH}_2\text{CH}_2\text{OH}$ ), 3.36 – 3.42 (m, 2H,  $\text{NCH}_2\text{CH}_2\text{OH}$ ), 3.89 – 4.01 (m, 8H,  $\text{OCH}_2\text{CH}_3$ ), 4.31 (br. s, 1H, OH) ppm.

#### Diethyl [3-(*N*-(2-hydroxyethyl)acetamido)propyl]phosphonate (**2.23a**)



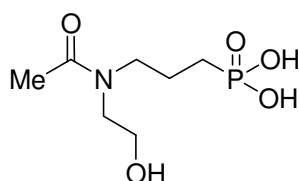
Amine **2.21** (500 mg, 2.0 mmol) was dissolved in a mixture of THF (20 mL) and 50% NaOAc solution (20 mL). Acetyl chloride (128  $\mu\text{L}$ , 1.80 mmol) was added dropwise over 30 min and the reaction mixture was stirred for 3 h at room temperature. Ethyl acetate (40 mL) was added and the phases were separated. The aqueous layer was extracted three times with ethyl acetate ( $3 \times 40$  mL) and the combined organic phase was dried over  $\text{MgSO}_4$ . After removal of the solvent under reduced pressure, the

mixture was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) yielding **26a** (390 mg, 70%) as a yellow oil.

**<sup>1</sup>H NMR** (300 MHz, DMSO-*d*<sub>6</sub>): mixture of amide rotamers  $\delta$  = 1.17 – 1.23 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.60 – 1.75 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 1.96 and 1.98 (2 × s, 3H, CH<sub>3</sub>C=O), 3.22 – 3.35 (m, 4H, CH<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>OH), 3.38 – 3.50 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>OH), 3.88 – 4.02 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.64 and 4.81 (2 × t, *J* = 5.1 Hz, 1H, OH) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>11</sub>H<sub>23</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 280.1312, found: 280.1324.

### [3-(*N*-(2-Hydroxyethyl)acetamido)propyl]phosphonic acid (**2.6**)



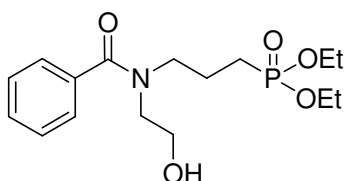
Trimethylsilyl bromide (1.12 g, 7.3 mmol) was added dropwise at 0 °C with stirring to a solution of phosphonic ester **2.23a** (390 mg, 1.39 mmol) in dichloromethane (8 mL), and stirring was continued at room temperature for 2 h. Organic solvents were removed *in vacuo*. The crude yellow foam was dissolved in ethanol and decolourized over a active carbon patch to yield the pure phosphonic acid **2.6** in almost quantitative yield.

**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 1.08 – 1.22 (m, 2H, CH<sub>2</sub>P), 1.48 – 1.65 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 1.96 and 1.97 (2 × s, 3H, CH<sub>3</sub>C=O), 3.14 – 3.26 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.30 – 3.38 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>OH), 3.51 – 3.60 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>OH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, D<sub>2</sub>O): mixture of amide rotamers  $\delta$  = 20.93 and 21.13 (CH<sub>3</sub>), 22.43 and 23.46 (d, <sup>2</sup>*J*<sub>C,P</sub> = 3.4 Hz, PCH<sub>2</sub>CH<sub>2</sub>), 26.50 and 26.71 (d, <sup>1</sup>*J*<sub>C,P</sub> = 131 Hz, PCH<sub>2</sub>), 47.93 and 50.58 (NCH<sub>2</sub>CH<sub>2</sub>OH), 47.48 and 51.45 (d, <sup>3</sup>*J*<sub>C,P</sub> = 19.9 Hz, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 59.19 and 59.24 (NCH<sub>2</sub>CH<sub>2</sub>OH), 174.59 and 174.72 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>7</sub>H<sub>15</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 224.0687, found: 224.0668.

### Diethyl [3-(*N*-(2-hydroxyethyl)benzamido)propyl]phosphonate (**2.23b**)

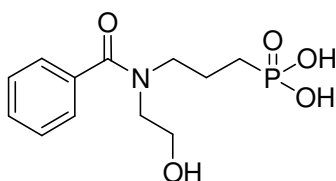


Amine **2.21** (334 mg, 1.4 mmol) was dissolved in a mixture of THF (20 mL) and 50% NaOAc solution (20 mL). Benzoyl chloride (147  $\mu$ L, 1.27 mmol) was added dropwise over 30 min and the reaction mixture was stirred for 3 h at room temperature. Ethyl acetate (40 mL) was added and the phases were separated. The aqueous layer was extracted three times with ethyl acetate (3  $\times$  40 mL) and the combined organic phase was dried over MgSO<sub>4</sub>. After removal of the solvent under reduced pressure, the mixture was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) yielding **2.23b** (383 mg, 79%) as a yellow oil.

**<sup>1</sup>H NMR** (300 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 1.22 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.20 – 1.78 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 3.23 – 3.60 (m, 6H, CH<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>OH), 3.98 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.75 (br. s, 1H, OH), 7.36 – 7.39 (m, 5H, arom. H) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>16</sub>H<sub>25</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 342.1469, found: 342.1486.

### [3-(*N*-(2-Hydroxyethyl)benzamido)propyl]phosphonic acid (**2.7**)



Trimethylsilyl bromide (897 mg, 5.85 mmol) was added dropwise at 0 °C with stirring to a solution of phosphonic ester **2.23b** (383 mg, 1.12 mmol) in dichloromethane (4 mL), and stirring was continued at room temperature for 2 h. Organic solvents were removed *in vacuo*. The crude yellow foam was dissolved in ethanol and decolourized over a active carbon patch to yield the pure phosphonic acid **2.7** in almost quantitative yield.

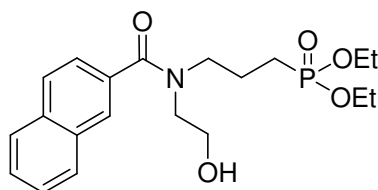
**<sup>1</sup>H NMR** (300 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 1.54 – 1.66 (m, 2H, CH<sub>2</sub>P), 1.80 – 1.89 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 3.04 – 3.09 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.35 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>OH), 4.49 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>OH), 7.33 – 8.09 (m, 5H, arom. H), 8.82 (br. s, 3H, OH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 20.48 (d, <sup>2</sup>J<sub>C,P</sub> = 4.0 Hz, PCH<sub>2</sub>CH<sub>2</sub>), 25.75 (d, <sup>1</sup>J<sub>C,P</sub> = 137.3 Hz, PCH<sub>2</sub>), 46.61 (NCH<sub>2</sub>CH<sub>2</sub>OH), 48.73 (d, <sup>3</sup>J<sub>C,P</sub> = 15.5 Hz, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 61.43 (NCH<sub>2</sub>CH<sub>2</sub>OH), 130.08 (=CH), 130.72 (=CH), 131.09 (=CH), 135.04 (=C), 167.21 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>12</sub>H<sub>17</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 286.0844, found: 286.0852.



### Diethyl [3-(*N*-(2-hydroxyethyl)-2-naphthamido)propyl]phosphonate (**2.23c**)

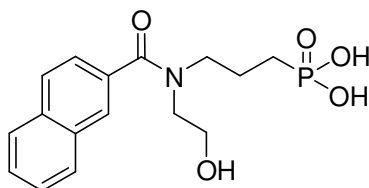


Amine **2.21** (358 mg, 1.5 mmol) was dissolved in a mixture of THF (20 mL) and 50% NaOAc solution (20 mL). 1-Naphthoyl chloride (204  $\mu$ L, 1.35 mmol) was added dropwise over 30 min and the reaction mixture was stirred for 3 h at room temperature. Ethyl acetate (40 mL) was added and the phases were separated. The aqueous layer was extracted three times with ethyl acetate (3  $\times$  40 mL) and the combined organic phase was dried over  $\text{MgSO}_4$ . After removal of the solvent under reduced pressure, the mixture was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5) yielding **2.23c** (487 mg, 82%) as a yellow oil.

**$^1\text{H}$  NMR** (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 1.05 (t,  $J$  = 7.0 Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.25 (t,  $J$  = 7.0 Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.32 – 1.60 (m, 2H,  $\text{CH}_2\text{CH}_2\text{P}$ ), 1.77 – 1.96 (m, 2H,  $\text{CH}_2\text{CH}_2\text{P}$ ), 2.95 – 3.21 (m, 2H,  $\text{NCH}_2$ ), 3.42 – 3.55 (m, 2H,  $\text{NCH}_2\text{CH}_2\text{OH}$ ), 3.69 – 3.78 (m, 2H,  $\text{NCH}_2\text{CH}_2\text{OH}$ ), 3.97 – 4.09 (m, 4H,  $\text{OCH}_2\text{CH}_3$ ), 4.63 and 4.86 (2  $\times$  br. s, 1H, OH), 7.39 – 7.99 (m, 7H, arom. H) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{20}\text{H}_{27}\text{NO}_5\text{P}$   $[\text{M}-\text{H}]^-$ : 392.1624, found: 392.1632.

### [3-(*N*-(2-Hydroxyethyl)-2-naphthamido)propyl]phosphonic acid (**2.8**)



Trimethylsilyl bromide (995 mg, 6.5 mmol) was added dropwise under Nitrogen at 0  $^\circ\text{C}$  with stirring to a solution of phosphonic ester **2.23c** (487 mg, 1.24 mmol) in dry dichloromethane (4 mL), and stirring was continued at room temperature for 2 h. Organic solvents were removed *in vacuo*. The crude yellow foam was taken up in acetone (6 mL) with vigorous shaking to result in a thin suspension. Water (0.25 mL) was added to give a gummy precipitate which solidified instantly. The suspension was shaken vigorously for 10 minutes, filtered and washed with acetone to give a

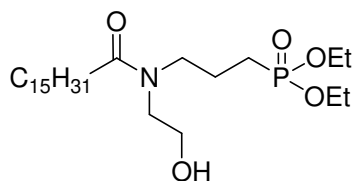
yellow solid compound. The solid was taken up in boiling water and the hot solution was filtered through a fluted filter paper to remove a small amount of insoluble material. The aqueous solution was cooled and crystallization began at once to yield the pure phosphonic acid **2.8**.

**<sup>1</sup>H NMR** (300 MHz, DMSO-*d*<sub>6</sub>): δ = 1.08 – 1.20 (m, 2H, CH<sub>2</sub>P), 1.56 – 1.67 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 3.02 – 3.09 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.30 – 3.49 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>OH), 3.72 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>OH), 4.58 (br. s, 1H, OH), 7.28 – 8.83 (m, 7H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, DMSO-*d*<sub>6</sub>): mixture of amide rotamers δ = 22.70 (d, <sup>2</sup>J<sub>C,P</sub> = 6.7 Hz, PCH<sub>2</sub>CH<sub>2</sub>), 24.91 (d, <sup>1</sup>J<sub>C,P</sub> = 138 Hz, PCH<sub>2</sub>), 38.56 (NCH<sub>2</sub>CH<sub>2</sub>OH), 48.11 (d, <sup>3</sup>J<sub>C,P</sub> = 19.3 Hz, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 61.55 (NCH<sub>2</sub>CH<sub>2</sub>OH), 125.34 (=CH), 125.86 (=CH), 127.26 (=CH), 127.32 (=CH), 127.51 (=CH), 128.00 (=CH), 128.45 (=CH), 131.64 (=C), 132.89 (=C), 133.60 (=C), 170.49 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>16</sub>H<sub>19</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 336.0999, found: 336.0990.

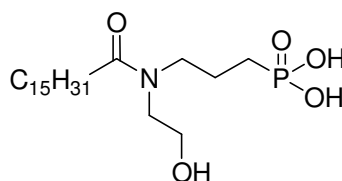
### Diethyl [3-(*N*-(2-hydroxyethyl)palmitamido)propyl]phosphonate (**2.23d**)



Amine **2.21** (240 mg, 1.0 mmol) was dissolved in a mixture of THF (20 mL) and 50% NaOAc solution (20 mL). Palmitoyl chloride (277 μL, 1.0 mmol) was added dropwise over 30 min and the reaction mixture was stirred for 3 h at room temperature. Ethyl acetate (40 mL) was added and the phases were separated. The aqueous layer was extracted three times with ethyl acetate (3 × 40 mL) and the combined organic phase was dried over MgSO<sub>4</sub>. After removal of the solvent under reduced pressure, the mixture was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) yielding **2.23d** (585 mg, 73%) as a yellow oil.

**<sup>1</sup>H NMR** (300 MHz, DMSO-*d*<sub>6</sub>): mixture of amide rotamers δ = 0.81 – 0.82 (m, 3H, CH<sub>3</sub>), 1.17 – 1.22 (m, 32H), 1.60 – 1.73 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>P), 2.21 – 2.31 (m, 2H, CH<sub>2</sub>C=O), 3.24 – 3.33 (m, 4H, CH<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>OH), 3.35 – 3.47 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>OH), 3.90 – 4.02 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.59 and 4.76 (2 × t, *J* = 5.1 Hz, 1H, OH) ppm.

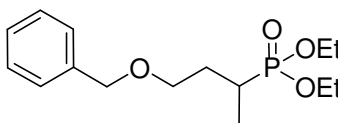
**Exact mass** (ESI-MS): calculated for C<sub>25</sub>H<sub>51</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 476.3503, found: 476.3517.

**[3-(*N*-(2-Hydroxyethyl)-2-palmitamido)propyl]phosphonic acid (2.9)**

Trimethylsilyl bromide (585 mg, 3.82 mmol) was added dropwise at 0 °C with stirring to a solution of phosphonic ester **2.23d** (348 mg, 0.73 mmol) in dichloromethane (4 mL), and stirring was continued at room temperature for 2 h. Organic solvents were removed *in vacuo*. The crude yellow foam was dissolved in ethanol and decolourized over a active carbon patch to yield the pure phosphonic acid **2.9** in almost quantitative yield.

**<sup>1</sup>H NMR** (300 MHz, DMSO-*d*<sub>6</sub>): δ = 0.96 (m, 3H, CH<sub>3</sub>), 1.29 (m, 30H), 2.25 (m, 2H), 3.50 (m, 6H) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>21</sub>H<sub>43</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 420.2878, found: 420.2893.

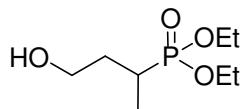
**Diethyl [4-(benzyloxy)butan-2-yl]phosphonate (2.25)**

In a three-necked flask of 250 mL was brought a 1.6 M solution of *n*BuLi in hexane (5.8 mL). At a temperature of -20 °C was added THF (5.8 mL) and the solution was cooled to -78 °C. Dropwise diethyl ethylphosphonate (1.55 g, 9.3 mmol) was added in THF (2 mL) by -78 °C. After 20 min benzyl 3-bromoethyl ether (1.47 mL, 9.3 mmol) was added in THF (4 mL). The reaction mixture was brought to room temperature and H<sub>2</sub>O was slowly added and the mixture was extracted four times with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 99:1) to give **2.25** in 28% yield.

**<sup>1</sup>H NMR** (300 MHz, DMSO-*d*<sub>6</sub>): δ = 0.99 and 1.05 (2 × d, *J* = 7.5 Hz, 3H, CH<sub>3</sub>), 1.18 (t, *J* = 7.0 Hz, 6 H, OCH<sub>2</sub>CH<sub>3</sub>), 1.36 – 1.52 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>CH), 1.84 – 2.02 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH), 3.40 – 3.54 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH), 3.90 – 4.20 (m, 4 H, OCH<sub>2</sub>CH<sub>3</sub>), 4.40 (1 H, d, AB system, *J* = 12.0 Hz, OCH<sub>2</sub>Ph), 4.46 (1 H, d, AB system, *J* = 12.0 Hz, OCH<sub>2</sub>Ph), 7.17 – 7.30 (m, 5 H, arom. H) ppm.

**Exact mass** (ESI-MS): calculated for  $C_{15}H_{26}O_4P$   $[M + H]^+$ : 301.1569, found 301.1573.

#### Diethyl [4-hydroxybutan-2-yl]phosphonate (**2.26**)

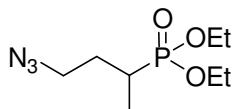


Phosphonate **2.25** (0.77 g, 2.6 mmol) was dissolved in MeOH (62 mL) and hydrogenated at atmospheric pressure in the presence of Pd (10 wt.-% on activated carbon, 843 mg). After stirring for 5 h, the reaction mixture was filtered through a Celite pad. The solvent was removed under vacuum, and the crude mixture was purified by column chromatography on silica gel **2.26** in 83% yield ( $CH_2Cl_2/MeOH$ , 99:1  $\rightarrow$  98:2  $\rightarrow$  97:3).

**$^1H$  NMR** (300 MHz,  $DMSO-d_6 + D_2O$ ):  $\delta$  = 0.99 and 1.06 (2  $\times$  d,  $J$  = 7.5 Hz, 3H,  $CH_3$ ), 1.40 (t,  $J$  = 7.0 Hz, 6 H,  $OCH_2CH_3$ ), 1.25 – 1.45 (m, 1H,  $CH_2CH_2CH$ ), 1.72 – 1.85 (m, 1H,  $CH_2CH_2CH$ ), 1.85 – 2.02 (m, 1H,  $CH_2CH_2CH$ ), 3.38 – 3.52 (m, 2H,  $CH_2CH_2CH$ ), 3.90 – 4.05 (m, 4 H,  $OCH_2CH_3$ ), 4.51 (t, 1H, OH) ppm.

**Exact mass** (ESI-MS): calculated for  $C_8H_{20}O_4P$   $[M + H]^+$ : 211.1099, found 211.1103.

#### Diethyl [4-azidobutan-2-yl]phosphonate (**2.28**)



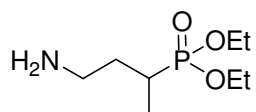
Methanesulfonylchloride (638 mg, 5.6 mmol) was added to a solution of compound **2.26** (300 mg, 1.4 mmol) in pyridine (8 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 20 h, and then the solvent was evaporated to dryness under reduced pressure. The residue was dissolved in  $CH_2Cl_2$  (150mL), washed with  $NaHCO_3$  solution and  $H_2O$ , and dried over anhydrous  $MgSO_4$ . The solvent was removed under diminished pressure to give a syrup (**2.27**) which was used in the following reaction without further purification. A solution of compound **2.27** and sodium azide (1.4 g, 21.4 mmol) in DMF (50 mL) was heated with stirring at 95 °C for 1.5 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue was dissolved in  $CH_2Cl_2$  (50 mL). The organic layer was washed with water (3  $\times$  20 mL), dried over anhydrous  $MgSO_4$ , and evaporated to give a syrup, which

was purified by silica gel column ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 95:5, v/v) to yield 0.19 g (56%) of product as a syrup.

**$^1\text{H}$  NMR** (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 0.99 and 1.52 (2  $\times$  d,  $J$  = 7.5 Hz, 3H,  $\text{CH}_3$ ), 1.40 (t,  $J$  = 7.0 Hz, 6 H,  $\text{OCH}_2\text{CH}_3$ ), 1.40 – 1.58 (m, 1H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 1.77 – 2.02 (m, 2H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 3.36 – 3.56 (m, 2H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 3.88 – 4.05 (m, 4 H,  $\text{OCH}_2\text{CH}_3$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_8\text{H}_{22}\text{N}_3\text{O}_3\text{P}$   $[\text{M} + \text{H}]^+$ : 239.1399, found 239.1406.

### Diethyl [4-aminobutan-2-yl]phosphonate (**2.29**)

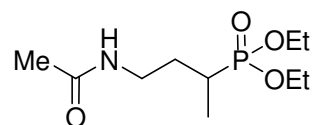


Compound **2.28** (190 mg, 0.79 mmol) and triphenylphosphine (333 mg, 1.27 mmol) were dissolved in pyridine (8 mL) and stirred at room temperature. After 1 h, concentrated ammonium hydroxide (8 mL) was added and the solution was allowed to stir for an additional 2 h. Pyridine was removed at reduced pressure, water (20 mL) was added, and the unreacted triphenylphosphine and triphenylphosphine oxide was removed by filtration. The filtrate was extracted with toluene and then water and concentrated to dryness to give a syrup which was purified by flash chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3$  in MeOH, 95:4:1, v/v/v) to yield 0.10 g (63%) of product **2.29** as a syrup.

**$^1\text{H}$  NMR** (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 0.99 and 1.05 (2  $\times$  d,  $J$  = 7.5 Hz, 3H,  $\text{CH}_3$ ), 1.21 (t,  $J$  = 7.0 Hz, 6 H,  $\text{OCH}_2\text{CH}_3$ ), 1.25 – 1.40 (m, 1H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 1.60 – 1.74 (m, 1H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 1.82 – 2.01 (m, 1H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 2.54 – 2.71 (m, 2H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 3.91 – 4.05 (m, 4 H,  $\text{OCH}_2\text{CH}_3$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_8\text{H}_{21}\text{NO}_3\text{P}$   $[\text{M} + \text{H}]^+$ : 210.1259, found 210.1244.

### Diethyl [4-acetamidobutan-2-yl]phosphonate (**2.30**)



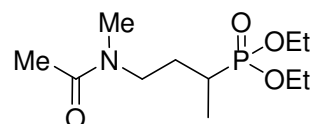
A solution of phosphonate **2.29** (103 mg, 0.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) containing pyridine (0.12 mL, 1.5 mmol) was cooled in an ice bath, followed by addition of acetylchloride (0.11 mL, 1.5 mmol). After 1.5 – 2 h the reaction was diluted with

CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and washed with water (50 mL). After drying of the organic layer with MgSO<sub>4</sub>, the solvent was removed *in vacuo* and the crude reaction mixture was chromatographed (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 99:1→96:4). Compound **2.30** was obtained as yellow oil in a yield of 86%.

**<sup>1</sup>H NMR** (300 MHz, DMSO-*d*<sub>6</sub>): δ = 1.00 and 1.06 (2 × d, *J* = 7.5 Hz, 3H, CH<sub>3</sub>), 1.20 (t, *J* = 7.0 Hz, 6 H, OCH<sub>2</sub>CH<sub>3</sub>), 1.22 – 1.33 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>CH), 1.69 – 1.88 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH), 1.76 (s, 3H, CH<sub>3</sub>C=O), 3.01 – 3.16 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH), 3.91 – 4.01 (m, 4 H, OCH<sub>2</sub>CH<sub>3</sub>), 7.82 (br. s, 1H, NH) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>10</sub>H<sub>23</sub>NO<sub>4</sub>P [M + H]<sup>+</sup>: 252.1365, found 252.1379.

### Diethyl [4-(N-methylacetamido)butan-2-yl]phosphonate (**2.31**)

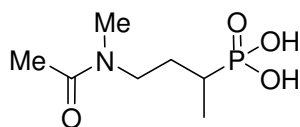


To a solution of phosphonate **2.30** (0.48 g, 1.9 mmol) in THF (10 mL) was added sodium hydride (57 mg, 2.3 mmol) in small portions. This reaction mixture was stirred by room temperature for 30 minutes. Iodomethane (0.35 g, 2.5 mmol) was added dropwise to the reaction mixture and the solution was stirred overnight. H<sub>2</sub>O was slowly added and the mixture was extracted four times with ethyl acetate. The organic layer was dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 99:1→98:2→97:3) to give **2.31** in 25% yield.

**<sup>1</sup>H NMR** (300 MHz, DMSO-*d*<sub>6</sub>): mixture of amide rotamers δ = 1.00 – 1.12 (m, 3H, H(C)CH<sub>3</sub>), 1.78 – 1.23 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.24 – 1.52 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>CH), 1.55 – 1.87 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH), 1.94 and 1.95 (2 × s, 3H, CH<sub>3</sub>C=O), 2.73 and 2.88 (2 × assymetric d, 3H, NCH<sub>3</sub>), 3.13 – 3.50 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH), 3.91 – 4.01 (m, 4 H, OCH<sub>2</sub>CH<sub>3</sub>) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>11</sub>H<sub>25</sub>NO<sub>4</sub>P [M + H]<sup>+</sup>: 266.1521, found 266.1532.

#### 4-(*N*-Methylacetamido)butan-2-ylphosphonic acid (**2.5**)



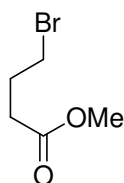
Trimethylsilyl iodide (343 mg, 1.7 mmol) was added dropwise at 0 °C with stirring to a solution of phosphonic ester **2.31** (111 mg, 0.42 mmol) in dichloromethane (10 mL), and stirring was continued at room temperature for 2 h. Organic solvents were removed *in vacuo*. The crude yellow foam was dissolved in ethanol, decolourized over a active carbon patch and subsequently crystallized from ethanol/Et<sub>2</sub>O to yield the pure phosphonic acid **2.5** in almost quantitative yield.

**<sup>1</sup>H-NMR** (300 MHz, D<sub>2</sub>O): mixture of amide rotamers  $\delta$  = 0.82 – 0.99 (m, 3H, H(C)CH<sub>3</sub>), 1.10 – 1.37 (m, 2H, CH<sub>2</sub>CHP), 1.72 – 1.99 (m, 1H, CH<sub>2</sub>CHP), 1.93 and 1.98 (2 × s, 3H, CH<sub>3</sub>C=O, minor and major form), 2.74 – 2.89 (2 × s, 3H, NCH<sub>3</sub>, major and minor form), 3.16 – 3.39 (m, 2H, NCH<sub>2</sub>) ppm.

**<sup>13</sup>C-NMR** (75 MHz, D<sub>2</sub>O): mixture of amide rotamers  $\delta$  = 14.15 and 14.32, 20.32 and 21.06, 28.81 and 29.80, 31.57, 33.62 and 36.21, 46.89 and 50.45, 173.9 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>7</sub>H<sub>15</sub>NO<sub>4</sub>P [M + H]<sup>+</sup>: 208.0737, found 208.0753.

#### Methyl $\gamma$ -bromobutyrate (**2.33**)



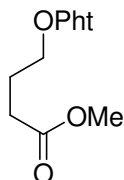
A solution of  $\gamma$ -butyrolactone (3 mL, 3.4 g, 39 mmol) in a 33% solution of hydrogen bromide in acetic acid (11.5 mL) was refluxed for 4 h at 75 °C, cooled to room temperature, treated with methanol (18 mL), and stirred overnight. Evaporation of the solvent gave a dark oil, which was dissolved in ethyl acetate (50 mL) and washed successively with saturated sodium bicarbonate (2 × 50 mL) and saturated sodium chloride (50 mL), dried over MgSO<sub>4</sub>, and evaporated to yield a clear oil (3.76 g, 53%).

**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 2.17 (quintet,  $J$  = 7.0 Hz, 2H, CH<sub>2</sub> $\beta$ ), 2.51 (t,  $J$  = 7.3 Hz, 2H, CH<sub>2</sub> $\alpha$ ), 3.47 (t,  $J$  = 6.5 Hz, 2H, CH<sub>2</sub> $\gamma$ ), 3.69 (s, 3H, OCH<sub>3</sub>).

**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 27.92, 32.41, 32.98, 51.99, 173.26.

**Exact mass** (ESI-MS): calculated for  $C_5H_{10}O_2Br$   $[M + H]^+$ : 180.9865, found 180.9892.

#### Methyl $\gamma$ -phthalimidooxybutyrate (2.34)



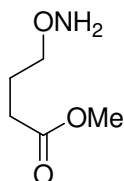
A mixture of methyl  $\gamma$ -bromobutyrate (3.75 g, 20.8 mmol), *N*-hydroxyphthalimide (3.4 g, 20.8 mmol), and triethylamine (5.86 mL, 4.22 g, 41.7 mol) in acetonitrile (30 mL) was refluxed for 3 h. The insoluble solid was removed by filtration, and the filtrate was evaporated. The residue was diluted with ethyl acetate (50 mL), and this solution was washed successively with water (3  $\times$  50 mL) and saturated sodium chloride (50 mL), dried over  $MgSO_4$ , and evaporated. The solid was recrystallized from hot ethanol to give 3.85 g (71%).

**$^1H$  NMR** (300 MHz,  $CDCl_3$ ):  $\delta$  = 2.09 (2H, quintet,  $J$  = 7.2 Hz,  $CH_2\beta$ ), 2.67 (2H, t,  $J$  = 7.3 Hz,  $CH_2\alpha$ ), 3.71 (3H, s,  $OCH_3$ ), 4.26 (t,  $J$  = 6.2 Hz, 2H,  $CH_2\gamma$ ), 7.74 – 7.85 (m, 4H, phthalimido ring).

**$^{13}C$  NMR** (75 MHz,  $CDCl_3$ ):  $\delta$  = 23.75, 30.20, 51.98, 77.67, 123.81, 129.11, 134.76, 163.87, 173.72.

**Exact mass** (ESI-MS): calculated for  $C_{13}H_{14}NO_5$   $[M + H]^+$ : 264.0872, found 264.0858

#### Methyl $\gamma$ -aminooxybutyrate (2.35)



A solution of methyl  $\gamma$ -phthalimidooxybutyrate (2.76 g, 10.49 mmol) in dichloromethane (50 mL) was cooled to  $-10$   $^{\circ}C$  and treated dropwise, with stirring, with methylhydrazine (0.86 mL, 0.74 g, 15.7 mmol). Stirring was continued for 1.5 h at  $-10$  to  $0$   $^{\circ}C$ , and the mixture was then filtered. The filtrate was concentrated, and the residue, in ethyl acetate (50 mL), was washed with 1:1 saturated sodium chloride:saturated sodium bicarbonate (20 mL). The aqueous layer was washed with



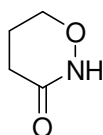
ethyl acetate (50 mL), and the combined organic extracts were dried over anhydrous magnesium sulfate and evaporated to give a yellow oil (1.44 g, 100%).

**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.86 (quintet, *J* = 6.2 Hz, 2H, CH<sub>2</sub>β), 2.32 (t, *J* = 7.3 Hz, 2H, CH<sub>2</sub>α), 3.62 (s, 3H, OCH<sub>3</sub>), 3.63 (t, *J* = 6.2 Hz, 2H, CH<sub>2</sub>γ).

**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 23.37, 30.91, 52.06, 76.08, 174.24.

**Exact mass** (ESI-MS): calculated for C<sub>5</sub>H<sub>12</sub>NO<sub>3</sub> [M + H]<sup>+</sup>: 134.0817, found 134.0728.

### [1,2]Oxazinan-3-one (2.36)



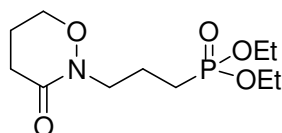
A solution of methyl γ-aminooxybutyrate (1.37 g, 10.4 mmol) in toluene (100 mL) was treated with a 2.0 mol/L solution of trimethylaluminium in hexanes (10.5 mL, 21 mmol). The resulting mixture was refluxed for 2 h, cooled to room temperature, and quenched with acetone (8 mL) with stirring for 30 min and then by slow addition of water (20 mL). Most of the solvent was removed under reduced pressure, and 1:4 dichloromethane:tetrahydrofuran (150 mL) was added. Filtration through Celite to remove aluminium salts and evaporation of the filtrate gave an oil which was purified by column chromatography (*n*-hexane/ethyl acetate, 1:7) (0.2 g, 20%).

**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 2.10 (quintet, *J* = 6.7 Hz, 2H, C=OCH<sub>2</sub>CH<sub>2</sub>), 2.52 (t, *J* = 7.3 Hz, 2H, C=OCH<sub>2</sub>), 4.02 (t, *J* = 6.2 Hz, 2H, CH<sub>2</sub>O).

**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 21.81, 27.86, 69.49, 163.24.

**Exact mass** (ESI-MS): calculated for C<sub>4</sub>H<sub>8</sub>NO<sub>2</sub> [M + H]<sup>+</sup>: 102.0555, found 102.0553.

### Diethyl [3-(3-oxomorpholino)propyl]phosphonate (2.37)



To a solution of **2.36** (46.5 mg, 0.46 mmol) in acetone (2 mL) was added potassium carbonate (0.13 g, 0.92 mmol). The suspension was heated at reflux for 1 h, then cooled at room temperature. Diethyl(3-bromo-propyl)phosphonate (**2.16**) (0.088 mL, 0.46 mmol) was hence added and, after refluxing for 12 h, the crude reaction mixture was diluted with acetone (10 mL) and poured into water (10 mL) and extracted with

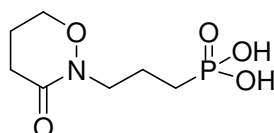
dichloromethane (3 × 10 mL). After the work up, the dark brown residue was chromatographed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95:5) to give **2.37** (57 mg, 43% yield).

**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.32 (t, *J* = 7.0 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.69 – 1.81 (m, 2H, PCH<sub>2</sub>), 1.85 – 1.99 (m, 2H, PCH<sub>2</sub>CH<sub>2</sub>), 2.09 (quintet, *J* = 7.0 Hz, 2H, C=OCH<sub>2</sub>CH<sub>2</sub>), 2.49 (t, *J* = 7.3 Hz, 2H, C=OCH<sub>2</sub>), 3.67 (t, *J* = 6.7 Hz, 2H, NCH<sub>2</sub>), 4.03 (t, *J* = 6.8 Hz, 2H, CH<sub>2</sub>O), 4.04 – 4.16 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>).

**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.70 (d, *J* = 6.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 20.64 (d, *J* = 4.6 Hz, PCH<sub>2</sub>CH<sub>2</sub>), 22.55 (C=OCH<sub>2</sub>CH<sub>2</sub>), 23.34 (d, *J* = 143.1 Hz, PCH<sub>2</sub>), 28.36 (CH<sub>2</sub>C=O), 46.42 (d, *J* = 19.9 Hz, NCH<sub>2</sub>), 61.82 (d, *J* = 6.6 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 69.21 (CH<sub>2</sub>O), 170.70 (C=O).

**Exact mass** (ESI-MS): calculated for C<sub>11</sub>H<sub>23</sub>NO<sub>5</sub>P [M + H]<sup>+</sup>: 280.1314, found 280.1299.

### 3-(3-Oxomorpholino)propylphosphonic acid (**2.38**)



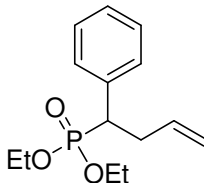
Trimethylsilyl bromide (125 mg, 0.8 mmol) was added dropwise with stirring to a solution of phosphonic ester **2.37** (57 mg, 0.2 mmol) in dichloromethane (2 mL), and stirring was continued at room temperature for 2 h. Organic solvents were removed *in vacuo*. The crude product was dissolved in H<sub>2</sub>O and purified with preparative HPLC on a C18 column (5 μm, Phenomenex, Luna, 250 × 21.2 mm) with a linear gradient of acetonitrile (0→100 %) in 5 mM NH<sub>4</sub>OAc solution at a flow rate of 17.5 mL/min over 20 min. The compound was detected by a UV-detector and ELSD-detector. The purity of the target compound was assessed by analytical HPLC [5 μm, Phenomenex, C18(2), 250 × 4.6 mm] using the same gradient at a flow rate of 1 mL/min. The phosphonic acid **2.38** was obtained as a yellow hygroscopic powder after lyophilisation (16 mg, 36% yield).

**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O): δ = 1.33 – 1.44 (m, 2H, PCH<sub>2</sub>), 1.60 – 1.71 (m, 2H, PCH<sub>2</sub>CH<sub>2</sub>), 1.92 (quintet, *J* = 6.7 Hz, 2H, C=OCH<sub>2</sub>CH<sub>2</sub>), 2.32 (t, *J* = 7.0 Hz, 2H, C=OCH<sub>2</sub>), 3.50 (t, *J* = 6.7 Hz, 2H, NCH<sub>2</sub>), 3.94 (t, *J* = 7.0 Hz, 2H, CH<sub>2</sub>O).

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O): δ = 20.98 (d, *J* = 3.7 Hz, PCH<sub>2</sub>CH<sub>2</sub>), 21.60 (C=OCH<sub>2</sub>CH<sub>2</sub>), 25.24 (d, *J* = 134.5 Hz, PCH<sub>2</sub>), 27.23 (CH<sub>2</sub>C=O), 47.42 (d, *J* = 19.9 Hz, NCH<sub>2</sub>), 69.93 (CH<sub>2</sub>O), 171.83 (C=O).

**Exact mass** (ESI-MS): calculated for  $C_7H_{15}NO_5P$   $[M + H]^+$ : 224.0688, found 224.0674.

**Diethyl (1-phenylbut-3-enyl)phosphonate (3.6a)**



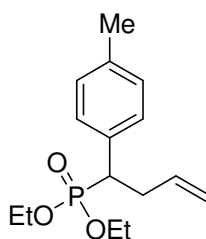
To a stirred solution of **3.5a** (12 mL, 57.4 mmol) in dry THF (100 mL) cooled to -50 to -70 °C, was added a 1.6 M solution of *n*BuLi (39 mL, 63.2 mmol) in hexane under  $N_2$ . After stirring at the same temperature for 15 min, allyl bromide (5 mL, 57.4 mmol) was added, 1 h after this addition, the reaction mixture was refluxed for 2 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo, and the resulting oil was diluted with toluene (200 mL), washed with 10 %  $NH_4Cl$  (200 mL) and water (200 mL), dried with  $MgSO_4$  and concentrated in vacuo. Purification of the residue by flash chromatography (*n*-hexane/ethyl acetate, 8:2→7:3→6:4) yielded compound **3.6a** as a transparent oil (14.96 g, 97 %).

**$^1H$  NMR** (300 MHz,  $CDCl_3$ ):  $\delta$  = 1.06 (t,  $J$  = 7.0 Hz, 3 H,  $OCH_2CH_3$ ), 1.25 (t,  $J$  = 7.0 Hz, 3 H,  $OCH_2CH_3$ ), 2.61 – 2.74 (m, 1 H, allyl  $CH_2$ ), 2.76 – 2.88 (m, 1 H, allyl  $CH_2$ ), 3.05 (ddd,  $J_{H,P}$  = 22.0 Hz,  $J$  = 4.4, 11.1 Hz, 1 H, CHP), 3.62 – 3.75 (m, 1 H,  $OCH_2CH_3$ ), 3.80 – 3.93 (m, 1 H,  $OCH_2CH_3$ ), 3.95 – 4.09 (m, 2 H,  $OCH_2CH_3$ ), 4.85 – 4.89 (m, 1 H,  $CH=CH_{2,cis}$ ), 4.93 – 5.00 (m, 1 H,  $CH=CH_{2,trans}$ ), 5.51 – 5.65 (m, 1 H,  $CH=CH_2$ ), 7.17 – 7.30 (m, 5 H, arom. H) ppm.

**$^{13}C$  NMR** (75 MHz,  $CDCl_3$ ):  $\delta$  = 16.46 (d,  $^3J_{C,P}$  = 5.7 Hz,  $OCH_2CH_3$ ), 16.63 (d,  $^3J_{C,P}$  = 6.0 Hz,  $OCH_2CH_3$ ), 34.26 (d,  $^2J_{C,P}$  = 2.9,  $CH_2CHP$ ), 44.81 (d,  $^1J_{C,P}$  = 137.1 Hz, CHP), 62.00 (d,  $^2J_{C,P}$  = 7.2 Hz,  $OCH_2CH_3$ ), 62.77 (d,  $^2J_{C,P}$  = 7.2 Hz,  $OCH_2CH_3$ ), 117.03, 127.34, 128.62, 129.55, 135.56, 135.82 ppm.

**Exact mass** (ESI-MS): calculated for  $C_{14}H_{22}O_3P$   $[M + H]^+$ : 269.1306, found 269.1292.

### Diethyl 1-*p*-tolylbut-3-enylphosphonate (3.6b)



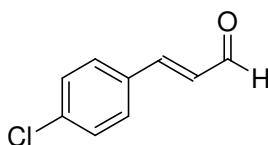
The procedure as described for **3.5a** was used to produce the title compound in a yield of 33 %.

**<sup>1</sup>H-NMR** (300 MHz, acetone-*d*<sub>6</sub>): δ = 1.06 (t, *J* = 7.3 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.25 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.29 (s, 3H, *p*-CH<sub>3</sub>), 2.56 – 2.68 (m, 1H, allyl CH<sub>2</sub>), 2.70 – 2.83 (m, 1H, allyl CH<sub>2</sub>), 3.10 (ddd, *J*<sub>H,P</sub> = 22.0 Hz, *J* = 4.1 Hz en *J* = 11.1 Hz, 1H, CHP), 3.65 – 3.78 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.79 – 3.92 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.96 – 4.09 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.83 – 4.88 (m, 1 H, CH=CH<sub>2</sub>, *cis*), 4.93 – 5.00 (m, 1 H, CH=CH<sub>2</sub>, *trans*), 5.56 – 5.69 (m, 1 H, CH=CH<sub>2</sub>), 7.11 – 7.14 (m, 2 H, arom. H), 7.22 – 7.25 (m, 2 H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, acetone-*d*<sub>6</sub>): δ = 15.94 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.5 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.12 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 20.38 (*p*-CH<sub>3</sub>), 34.35 (d, <sup>2</sup>*J*<sub>C,P</sub> = 2.6, CH<sub>2</sub>CHP), 43.80 (d, <sup>1</sup>*J*<sub>C,P</sub> = 137.4 Hz, CHP), 61.38 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 61.99 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 116.11, 128.99, 129.58, 133.40, 136.15, 136.43 ppm.

**Mass** (ESI-MS): calculated for C<sub>15</sub>H<sub>24</sub>O<sub>3</sub>P [M+H]<sup>+</sup>: 283.1462, found: 283.1.

### (*E*)-3-(4-chlorophenyl)acrylaldehyde (3.7d)



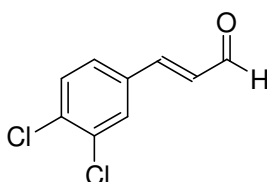
To a stirred solution of 1-chloro-4-iodobenzene (2 g, 8.39 mmol) in DMF (30 mL) were added acrolein diethyl acetal (3.84 mL, 8.39 mmol), <sup>n</sup>Bu<sub>4</sub>NOAc (5.06 g, 16.8 mmol), K<sub>2</sub>CO<sub>3</sub> (1.74 g, 12.6 mmol), KCl (0.63 g, 8.39 mmol) and Pd(OAc)<sub>2</sub> (0.056 g, 0.25 mmol). The mixture was stirred for 1.5 h at 90 °C. After cooling, 2 N HCl was slowly added and the reaction mixture was stirred at room temperature for 10 min. Subsequently, it was diluted with ether (200 mL) and washed with water (200 mL). The organic layer was dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by flash chromatography (*n*-hexane/ethylacetate 8:2) to give **3.7d** in 74% yield.

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 6.67 (dd, *J* = 7.6 and 16.1 Hz, 1H, *CHC*(O)H), 7.42 (d, *J* = 16.1 Hz, 1H, *CH=CHC*(O)H), 7.44 (m, 4H, arom. H), 9.69 (d, *J* = 7.6 Hz, 1 H, C(O)H) ppm.

**<sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>)**: δ = 129.19 (=CH), 129.65 (=CH), 129.83 (=CH), 132.73 (=C), 137.48 (=C), 151.20 (=CH), 193.53 (C=O) ppm.

**Mass** (ESI-MS): calculated for C<sub>9</sub>H<sub>8</sub>ClO [M+H]<sup>+</sup>: 167.0264, found: 167.1.

**(*E*)-3-(3,4-dichlorophenyl)acrylaldehyde (3.7e)**



A synthetic procedure as described for **3.7d** using 1,2-dichloro-4-iodobenzene gave 58% of the title compound.

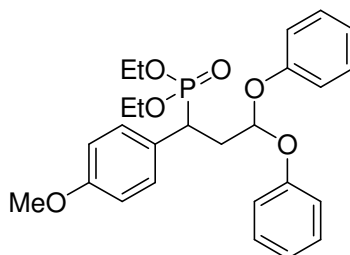
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 6.68 (dd, *J* = 7.3 and 16.1 Hz, 1H, *CHC*(O)H), 7.37 (d, *J* = 16.1 Hz, 1H, *CH=CHC*(O)H), 7.45 (m, 3H, arom. H), 9.71 (d, *J* = 7.3 Hz, 1 H, C(O)H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 127.42 (=CH), 130.12 (=CH), 130.26 (=CH), 131.36 (=CH), 133.80 (=C), 134.22 (=C), 135.48 (=C), 149.56 (=CH), 193.14 (C=O) ppm.

**Mass** (ESI-MS): calculated for C<sub>9</sub>H<sub>7</sub>Cl<sub>2</sub>O [M+H]<sup>+</sup>: 200.9874, found: 200.1.

**General Method for the Synthesis of 3.8c-e:** A mixture of the appropriate acrylic aldehyde (6.17 mmol), triethyl phosphite (1.34 mL, 7.71 mmol) and phenol (1.54 g, 16 mmol) was heated to 100 °C. After 24 h, TLC analysis (hexane/ethyl acetate, 6:4) indicated that the reaction was finished, and the reaction mixture was subsequently concentrated. The crude product was purified by flash chromatography, eluting with hexane/ethyl acetate, 6:4. After concentration of the pure fractions, the desired acetals **3.8c-e** were obtained as slightly yellow oils.

**Diethyl [1-(4-methoxyphenyl)-3,3-diphenoxypropyl]phosphonate (3.8c).** Yield: 81%.



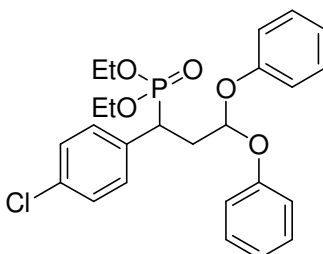
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.08 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.25 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.45 – 2.59 (m, 1H, PCHCH<sub>2</sub>), 2.68 – 2.79 (m, 1H, PCHCH<sub>2</sub>), 3.36 (ddd, *J*<sub>H,P</sub> = 22.4 Hz, *J* = 4.6 Hz and *J* = 10.9 Hz, 1H, CHP), 3.64 – 3.75 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.80 (s, 3H, OCH<sub>3</sub>), 3.83 – 3.96 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.97 – 4.10 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 5.61 (dd, *J* = 7.3 Hz and *J* = 3.8 Hz, 1H, CH(OPh)<sub>2</sub>), 6.81 – 6.92 (m, 6H, arom. H), 6.95 – 7.02 (m, 1H, arom. H), 7.18 – 7.30 (m, 7H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.45 (d, <sup>3</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.53 (d, <sup>3</sup>*J*<sub>C,P</sub> = 6.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 34.89 (PCHCH<sub>2</sub>), 39.51 (d, <sup>1</sup>*J*<sub>C,P</sub> = 140.2 Hz, CHP), 55.31 (OCH<sub>3</sub>), 62.07 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.93 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 99.60 (d, <sup>3</sup>*J*<sub>C,P</sub> = 16.7 Hz, CH(OPh)<sub>2</sub>), 114.36 (d, =CH), 117.58 (=CH), 117.73 (=CH), 122.68 (=CH), 122.81 (=CH), 127.15 (d, =C), 129.71 (=CH), 129.72 (=CH), 130.44 (d, =CH), 156.18 (=C), 156.31 (=C), 159.22 (d, =C) ppm.

**<sup>31</sup>P-NMR** (120 MHz, CDCl<sub>3</sub>): δ = 29.09 ppm.

**Mass** (ESI-MS): calculated for C<sub>26</sub>H<sub>32</sub>O<sub>6</sub>P [M+H]<sup>+</sup>: 471.1937, found: 471.2.

**Diethyl [1-(4-chlorophenyl)-3,3-diphenoxypropyl]phosphonate (3.8d).** Yield: 85%.



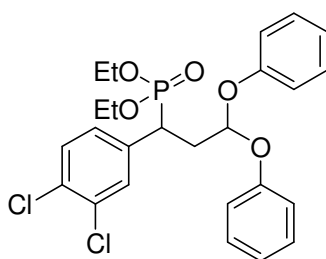
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.09 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.25 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.46 – 2.60 (m, 1H, PCHCH<sub>2</sub>), 2.71 – 2.83 (m, 1H, PCHCH<sub>2</sub>), 3.41 (ddd, *J*<sub>H,P</sub> = 22.6 Hz, *J* = 4.7 Hz and *J* = 10.6 Hz, 1H, CHP), 3.67 – 3.80 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.85 – 3.96 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.98 – 4.11 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 5.63

(ddd,  $J = 7.3$  Hz and  $J = 4.4$  Hz,  $J_{\text{H,P}} = 0.88$  Hz, 1H,  $\text{CH}(\text{OPh})_2$ ), 6.81 – 6.92 (m, 4H, arom. H), 6.96 – 7.02 (m, 2H, arom. H), 7.18 – 7.26 (m, 8H, arom. H) ppm.

**$^{13}\text{C}$ -NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 16.46$  (d,  $^3J_{\text{C,P}} = 5.8$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 16.56 (d,  $^3J_{\text{C,P}} = 6.1$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 34.70 (PCHCH<sub>2</sub>), 39.88 (d,  $^1J_{\text{C,P}} = 139.9$  Hz, CHP), 62.39 (d,  $^2J_{\text{C,P}} = 7.5$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 63.13 (d,  $^2J_{\text{C,P}} = 7.2$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 99.47 (d,  $^3J_{\text{C,P}} = 16.7$  Hz,  $\text{CH}(\text{OPh})_2$ ), 117.67 (=CH), 117.73 (=CH), 122.91 (d, =CH), 129.13 (=CH), 129.16 (=CH), 129.79 (=CH), 129.81 (=CH), 130.77 (d, =CH), 133.67 (=C), 134.24 (=C), 156.09 (=C), 156.11 (=C) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{25}\text{H}_{28}\text{ClO}_5\text{PNa}$   $[\text{M}+\text{Na}]^+$ : 497.1260, found: 497.1261.

**Diethyl [1-(3,4-Dichlorophenyl)-3,3-diphenoxypropyl]phosphonate (3.8e).** Yield: 2.29 g (70%).

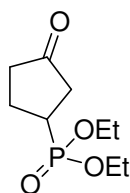


**$^1\text{H}$  NMR** (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 1.13$  (t,  $J = 7.0$  Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.25 (t,  $J = 7.0$  Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 2.43 – 2.57 (m, 1H, PCHCH<sub>2</sub>), 2.70 – 2.82 (m, 1H, PCHCH<sub>2</sub>), 3.38 (ddd,  $J_{\text{H,P}} = 22.7$  Hz,  $J = 4.7, 10.3$  Hz, 1H, CHP), 3.74 – 3.87 (m, 1H,  $\text{OCH}_2\text{CH}_3$ ), 3.89 – 3.99 (m, 1H,  $\text{OCH}_2\text{CH}_3$ ), 3.99 – 4.12 (m, 2H,  $\text{OCH}_2\text{CH}_3$ ), 5.66 [dd,  $J = 6.8, 4.4$  Hz, 1H,  $\text{CH}(\text{OPh})_2$ ], 6.84 – 6.92 (m, 3H, arom. H), 6.97 – 7.03 (m, 2H, arom. H), 7.18 – 7.27 (m, 6H, arom. H), 7.37 – 7.45 (m, 2H, arom. H) ppm.

**$^{13}\text{C}$  NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 16.51$  (app. t,  $^3J_{\text{C,P}} = 5.8$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 34.61 (d,  $^2J_{\text{C,P}}$  too small for detection, PCHCH<sub>2</sub>), 39.70 (d,  $^1J_{\text{C,P}} = 139.9$  Hz, CHP), 62.57 (d,  $^2J_{\text{C,P}} = 7.2$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 63.16 (d,  $^2J_{\text{C,P}} = 6.6$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 99.34 [d,  $^3J_{\text{C,P}} = 15.8$  Hz,  $\text{CH}(\text{OPh})_2$ ], 117.66 (arom. C), 117.68 (arom. C), 122.99 (arom. C), 123.01 (arom. C), 128.75 (d, arom. C), 129.83 (arom. C), 129.85 (arom. C), 130.81 (d, arom. C), 131.29 (d, arom. C), 131.87 (d, arom. C), 133.01 (d, arom. C), 136.27 (d, arom. C), 155.95 (arom. C), 156.04 (arom. C) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{25}\text{H}_{27}\text{Cl}_2\text{O}_5\text{PNa}$   $[\text{M} + \text{Na}]^+$  531.0871, found 531.0872.

**Diethyl 3-oxocyclopentylphosphonate (3.19).** Yield: 90%.



**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.31 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.32 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.02 – 2.56 (m, 7H, C<sub>5</sub>H<sub>7</sub>P), 4.05 – 4.19 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.73 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.5 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 23.74 (d, *J*<sub>C,P</sub> = 3.7 Hz, CH<sub>2</sub>), 33.11 (d, <sup>1</sup>*J*<sub>C,P</sub> = 153.5 Hz, CHP), 37.89 (d, *J*<sub>C,P</sub> = 7.9 Hz, CH<sub>2</sub>), 38.97 (d, *J*<sub>C,P</sub> = 2.9 Hz, CH<sub>2</sub>), 62.25 (app t, <sup>2</sup>*J*<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 216.66 (d, <sup>3</sup>*J*<sub>C,P</sub> = 15.5 Hz, C=O) ppm.

**<sup>31</sup>P-NMR** (120 MHz, CDCl<sub>3</sub>): δ = 31.66 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>9</sub>H<sub>18</sub>O<sub>4</sub>P [M+H]<sup>+</sup>: 221.0943, found: 221.0936.

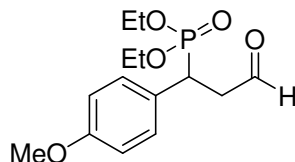
**General Method for the Synthesis of 3.9a,b:** To a mixture of alkene **3.6a** or **3.6b** (6.56 mmol) and 4-methylmorpholine *N*-oxide (0.92 g, 7.87 mmol) in dioxane (40 mL) was added an aqueous 1% solution of OsO<sub>4</sub> (99.1 mg, 0.39 mmol). After stirring with protection from light at room temperature overnight, the starting material was completely converted according to TLC. Sodium periodate (2.24 g, 10.5 mmol) was then added in small portions. After completion of the reaction (2 h), the mixture was diluted with ethyl acetate (100 mL), filtered through Celite, and solids were washed with ethyl acetate. The combined filtrates were washed with saturated aqueous NaCl (100 mL), dried with MgSO<sub>4</sub>, and the solvents were evaporated under vacuum to yield crude **3.9a** or **3.9b**, which were used in the next step without further purification.

**General Method for Synthesis of 3.9c-e:** Acetals **3.8c-e** (5.0 mmol) were hydrolyzed by treatment with a mixture of water (7 mL), acetone (35 mL) and 2 N HCl (8 mL). After heating to 60-70 °C for 3-4 h, TLC analysis (ethyl acetate) confirmed that the reaction was finished. The solvents were evaporated under vacuum, and the residue was dissolved in ethyl acetate (200 mL) and transferred to a separating funnel, where it was washed twice with water (200 mL). The organic layer was dried with MgSO<sub>4</sub> and the solvents were evaporated. The residue was purified by flash chromatography using ethyl acetate as eluent to yield **3.9c-e** as transparent oils.



NMR revealed, by disappearance of the CH=O signals, that **3.9a-e** are prone to oxidation upon storage when dissolved in CDCl<sub>3</sub>.

**Diethyl [2-formyl-1-(4-methoxyphenyl)ethyl]phosphonate (3.9c).** Yield: 77%.



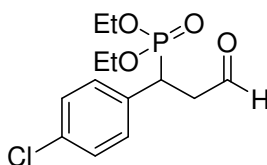
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.12 (t,  $J$  = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.27 (t,  $J$  = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.99 – 3.20 (m, 2H, PCHCH<sub>2</sub>), 3.66 (ddd,  $J_{H,P}$  = 22.6 Hz,  $J$  = 5.3 Hz and  $J$  = 9.1 Hz, 1H, CHP), 3.71 – 3.80 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.78 (s, 3H, OCH<sub>3</sub>), 3.82 – 3.95 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.97– 4.09 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 6.83 – 6.86 (m, 2H, arom. H), 7.25 – 7.29 (m, 2H, arom. H), 9.66 (app q, 1H, HC=O) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 16.57 (app t,  $^3J_{C,P}$  = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 37.32 (d,  $^1J_{C,P}$  = 142.2 Hz, CHP), 44.34 (PCHCH<sub>2</sub>), 55.46 (OCH<sub>3</sub>), 62.36 (d,  $^2J_{C,P}$  = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.16 (d,  $^2J_{C,P}$  = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 114.33 (d,  $^4J_{C,P}$  = 2.3 Hz, =C<sub>m</sub>H), 127.14 (d,  $^2J_{C,P}$  = 7.2 Hz, =C<sub>i</sub>), 130.35 (d,  $^3J_{C,P}$  = 6.6 Hz, =C<sub>o</sub>H), 159.18 (d,  $^5J_{C,P}$  = 3.2 Hz, =C<sub>p</sub>), 199.41 (d,  $^3J_{C,P}$  = 15.8 Hz, HC=O) ppm.

**<sup>31</sup>P-NMR** (120 MHz, CDCl<sub>3</sub>):  $\delta$  = 28.49 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>14</sub>H<sub>22</sub>O<sub>5</sub>P [M+H]<sup>+</sup>: 301.1205, found: 301.1206.

**Diethyl [1-(4-chlorophenyl)-2-formylethyl]phosphonate (3.9d).** Yield: 83%.



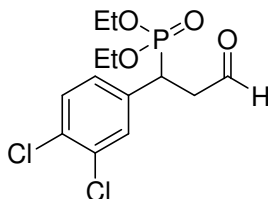
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.13 (t,  $J$  = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.27 (t,  $J$  = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 3.00 – 3.23 (m, 2H, PCHCH<sub>2</sub>), 3.69 (ddd,  $J_{H,P}$  = 22.7 Hz,  $J$  = 4.7 Hz and  $J$  = 9.4 Hz, 1H, CHP), 3.72 – 3.82 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.83– 3.96 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.97– 4.09 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 7.28 (s, 4H, arom. H), 9.65 – 9.66 (m, 1H, HC=O) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 16.43 (app t,  $^3J_{C,P}$  = 6.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 37.30 (d,  $^1J_{C,P}$  = 142.2 Hz, CHP), 44.01 (PCHCH<sub>2</sub>), 62.84 (d,  $^2J_{C,P}$  = 7.5 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.44 (d,  $^2J_{C,P}$  = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 129.06 (d,  $^4J_{C,P}$  = 2.6 Hz, =C<sub>m</sub>H), 130.63 (d,  $^3J_{C,P}$  = 6.3

Hz, =C<sub>o</sub>H), 133.75 (d, <sup>5</sup>J<sub>C,P</sub> = 5.5 Hz, =C<sub>p</sub>), 133.92 (d, <sup>2</sup>J<sub>C,P</sub> = 7.2 Hz, =C<sub>i</sub>), 198.55 (d, <sup>3</sup>J<sub>C,P</sub> = 15.8 Hz, HC=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>13</sub>H<sub>18</sub>ClO<sub>4</sub>P [M+H]<sup>+</sup>: 305.0710, found: 305.0702.

**Diethyl [1-(3,4-dichlorophenyl)-2-formylethyl]phosphonate (3.9e).** Yield: 1.28 g (76%).



**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.11 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.23 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.95 – 3.20 (m, 2H, PCHCH<sub>2</sub>), 3.62 (ddd, *J*<sub>H,P</sub> = 22.7 Hz, *J* = 4.7, 9.7 Hz, 1H, CHP), 3.74 – 3.83 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.84 – 3.95 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.96 – 4.07 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 7.12 – 7.40 (m, 3H, arom. H), 9.61 – 9.62 (m, 1H, HC=O) ppm.

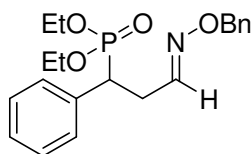
**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.54 (app. t, <sup>3</sup>J<sub>C,P</sub> = 6.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 37.20 (d, <sup>1</sup>J<sub>C,P</sub> = 141.9 Hz, CHP), 44.09 (d, <sup>2</sup>J<sub>C,P</sub> = 2.3 Hz, PCHCH<sub>2</sub>), 62.75 (d, <sup>2</sup>J<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.34 (d, <sup>2</sup>J<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 128.70 (d, <sup>3</sup>J<sub>C,P</sub> = 6.3 Hz, arom. C<sub>o</sub>), 130.75 (d, <sup>4</sup>J<sub>C,P</sub> = 2.6 Hz, arom. C<sub>m</sub>), 131.15 (d, <sup>3</sup>J<sub>C,P</sub> = 6.9 Hz, arom. C<sub>o</sub>), 131.95 (d, <sup>5</sup>J<sub>C,P</sub> = 3.7 Hz, arom. C<sub>p</sub>), 132.92 (d, <sup>4</sup>J<sub>C,P</sub> = 2.9 Hz, arom. C<sub>m</sub>), 136.03 (d, <sup>2</sup>J<sub>C,P</sub> = 7.2 Hz, arom. C<sub>i</sub>), 198.13 (d, <sup>3</sup>J<sub>C,P</sub> = 15.0 Hz, HC=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>13</sub>H<sub>18</sub>Cl<sub>2</sub>O<sub>4</sub>P [M + H]<sup>+</sup> 339.03204, found 339.0325.

**General Method for the Synthesis of 3.10a-e and 3.20:** A mixture of aldehydes **3.9a-e** (3.86 mmol) and *O*-benzylhydroxylamine hydrochloride (0.61 g, 3.86 mmol) in pyridine/ethanol, 1:1 (14 mL) was stirred at room temperature under nitrogen for 1.5-6 h. After the solvent was removed by evaporation, the residue was co-evaporated three times with toluene and subsequently purified by chromatography on a silica gel column (*n*-hexane/ethyl acetate, 6:4 or 6:4→1:1) to give a mixture of benzyloxyimines **3.10a-e** as transparent oils.

**Diethyl [(E)- and (Z)-3-(benzyloxy)imino-1-phenylpropylphosphonate (3.10a).**

Yield: 82% from **3.6a**.



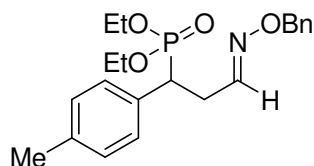
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.09 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.27 (dt, *J* = 7.0 Hz, *J*<sub>H,P</sub> = 4.4 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.79 – 3.15 (m, 2H, CHPCCH<sub>2</sub>), 3.16 – 3.33 (m, 1H, PCH), 3.64 – 3.78 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.82 – 3.94 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.95 – 4.10 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.98 (s, 1H, OCH<sub>2</sub>Ph), 5.08 (s, 1H, OCH<sub>2</sub>Ph), 6.56 (t, *J* = 5.0 Hz, 1H, HC=N), 7.24 – 7.33 (m, 10H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.46 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.63 (d, <sup>3</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 26.68 (br d, CH<sub>2</sub>CHP), 30.33 (d, <sup>2</sup>*J*<sub>C,P</sub> = 2.3 Hz, CH<sub>2</sub>CHP), 41.73 (d, <sup>1</sup>*J*<sub>C,P</sub> = 139.8 Hz, CHP), 42.55 (d, <sup>1</sup>*J*<sub>C,P</sub> = 139.1 Hz, CHP), 62.26 (d, <sup>2</sup>*J*<sub>C,P</sub> = 4.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.36 (d, <sup>2</sup>*J*<sub>C,P</sub> = 4.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.00 (d, <sup>2</sup>*J*<sub>C,P</sub> = 2.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.09 (d, <sup>2</sup>*J*<sub>C,P</sub> = 2.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 75.87 (OCH<sub>2</sub>Ph), 76.14 (OCH<sub>2</sub>Ph), 127.75 (arom. C), 128.03 (arom. C), 128.28 (arom. C), 128.60 (arom. C), 128.85 (arom. C), 129.43 (arom. C), 135.11 (arom. C), 137.89 (arom. C), 148.83 (d, <sup>3</sup>*J*<sub>C,P</sub> = 16.7 Hz, HC=N), 149.37 (d, <sup>3</sup>*J*<sub>C,P</sub> = 15.8 Hz, HC=N) ppm. (most aromatic peaks are double due to coupling with P or because of the presence of two isomers)

**Exact mass** (ESI-MS): calculated for C<sub>20</sub>H<sub>27</sub>NO<sub>4</sub>P [M+H]<sup>+</sup>: 376.1678, found: 376.1670.

**Diethyl [(E)- and (Z)-3-(benzyloxy)imino-1-*p*-tolylpropylphosphonate (3.10b).**

Yield: 82% from **3.6b**.

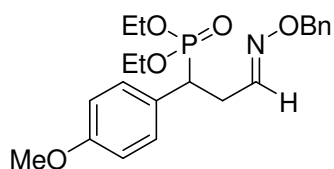


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.05 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.20 (dt, *J* = 7.0 Hz, *J*<sub>H,P</sub> = 4.4 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.25 (s, 3H, *p*-CH<sub>3</sub>), 2.69 – 3.05 (m, 2H, CHPCCH<sub>2</sub>), 3.06 – 3.22 (m, 1H, PCH), 3.59 – 3.74 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.77– 3.88 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.90 – 4.03 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.92 (s, 1H, OCH<sub>2</sub>Ph), 5.02 (s, 1H, OCH<sub>2</sub>Ph), 6.49 (t, *J* = 5.0 Hz, 1H, HC=N), 7.02 – 7.29 (m, 9H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 15.24 (d, <sup>3</sup>J<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 15.39 (d, <sup>3</sup>J<sub>C,P</sub> = 6.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 20.08 (*p*-CH<sub>3</sub>), 25.46 (d, CH<sub>2</sub>CHP), 29.08 (d, CH<sub>2</sub>CHP), 40.04 (d, <sup>1</sup>J<sub>C,P</sub> = 139.6 Hz, CHP), 40.88 (d, <sup>1</sup>J<sub>C,P</sub> = 139.3 Hz, CHP), 60.99 (d, <sup>2</sup>J<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 61.06 (d, <sup>2</sup>J<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 61.70 (d, <sup>2</sup>J<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 61.73 (d, <sup>2</sup>J<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 74.57 (OCH<sub>2</sub>Ph), 74.85 (OCH<sub>2</sub>Ph), 126.74 (arom. C), 127.01 (arom. C), 127.32 (arom. C), 127.99 (arom. C), 128.32 (arom. C), 130.63 (arom. C), 136.10 (arom. C), 136.68 (arom. C), 147.76 (d, <sup>3</sup>J<sub>C,P</sub> = 17.0 Hz, HC=N), 148.25 (d, <sup>3</sup>J<sub>C,P</sub> = 15.8 Hz, HC=N) ppm. (most aromatic peaks are double due to coupling with P or because of the presence of two isomers)

**Mass** (ESI-MS): calculated for C<sub>21</sub>H<sub>29</sub>NO<sub>4</sub>P [M+H]<sup>+</sup>: 390.1834, found: 390.2.

**Diethyl [(*E*)- and (*Z*)-3-(benzyloxy)imino-1-(4-methoxyphenyl)propylfosfonaat (3.10c).** Yield: 67%.

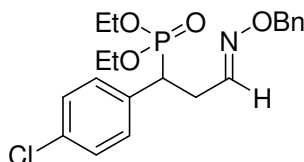


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.11 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.26 (dt, *J* = 7.0 Hz, *J*<sub>H,P</sub> = 4.4 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.73 – 3.11 (m, 2H, CHPCH<sub>2</sub>), 3.12 – 3.27 (m, 1H, PCH), 3.66 – 3.80 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.77 and 3.83 (2 x s, 3H, OCH<sub>3</sub>, (*E*) and (*Z*)-isomers), 3.82– 3.94 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.96– 4.08 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.98 (s, 1H, OCH<sub>2</sub>Ph), 5.08 (s, 1H, OCH<sub>2</sub>Ph), 6.55 (t, *J* = 5.0 Hz, 1H, HC=N), 6.80 – 6.85 (m, 2H, arom. H), 7.19 – 7.34 (m, 7H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.52 (d, <sup>3</sup>J<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.64 (d, <sup>3</sup>J<sub>C,P</sub> = 5.6 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 26.75 (d, CH<sub>2</sub>CHP), 30.42 (d, CH<sub>2</sub>CHP), 40.83 (d, <sup>1</sup>J<sub>C,P</sub> = 140.2 Hz, CHP), 41.64 (d, <sup>1</sup>J<sub>C,P</sub> = 139.9 Hz, CHP), 55.43 (OCH<sub>3</sub>), 62.20 (d, <sup>2</sup>J<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.27 (d, <sup>2</sup>J<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.94 (d, <sup>2</sup>J<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.98 (d, <sup>2</sup>J<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 75.81 (OCH<sub>2</sub>Ph), 76.11 (OCH<sub>2</sub>Ph), 114.27 (=CH), 126.72 – 127.11 (=C), 127.96 and 128.00 (=CH), 128.21 and 128.29 (=CH), 128.55 and 128.58 (=CH), 130.32 – 130.55 (=CH), 137.84 and 138.102 (=C), 148.94 (d, <sup>3</sup>J<sub>C,P</sub> = 17.3 Hz, HC=N), 149.46 (d, <sup>3</sup>J<sub>C,P</sub> = 16.1 Hz, HC=N), 159.10 – 159.21 (=C) ppm. (most aromatic peaks are double due to coupling with P or because of the presence of two isomers)

**Exact mass** (ESI-MS): calculated for  $C_{21}H_{29}NO_5P$   $[M+H]^+$ : 406.1783, found: 406.1793.

**Diethyl [(E)- and (Z)-3-(benzyloxy)imino-1-(4-chlorophenyl)propylphosphonate (3.10d).** Yield: 85%.

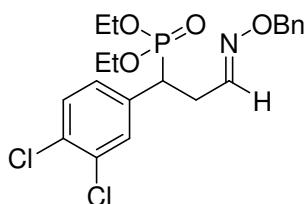


**$^1H$ -NMR** (300 MHz,  $CDCl_3$ ):  $\delta$  = 1.13 (t,  $J$  = 7.0 Hz, 3H,  $OCH_2CH_3$ ), 1.27 (dt,  $J$  = 7.0 Hz,  $J_{H,P}$  = 3.8 Hz, 3H,  $OCH_2CH_3$ ), 2.74 – 3.08 (m, 2H,  $CHPCH_2$ ), 3.18 – 3.32 (m, 1H, PCH), 3.74 – 3.85 (m, 1H,  $OCH_2CH_3$ ), 3.86 – 3.96 (m, 1H,  $OCH_2CH_3$ ), 3.97 – 4.09 (m, 2H,  $OCH_2CH_3$ ), 4.97 (s, 1H,  $OCH_2Ph$ ), 5.08 (s, 1H,  $OCH_2Ph$ ), 6.54 (t,  $J$  = 5.0 Hz, 1H,  $HC=N$ ), 7.19 – 7.35 (m, 9H, arom. H) ppm.

**$^{13}C$ -NMR** (75 MHz,  $CDCl_3$ ):  $\delta$  = 16.51 (d,  $^3J_{C,P}$  = 5.8 Hz,  $OCH_2CH_3$ ), 16.62 (d,  $^3J_{C,P}$  = 6.9 Hz,  $OCH_2CH_3$ ), 26.60 (d,  $^2J_{C,P}$  = 2.0 Hz,  $CH_2CHP$ ), 30.26 (d,  $^2J_{C,P}$  = 2.0 Hz,  $CH_2CHP$ ), 41.19 (d,  $^1J_{C,P}$  = 139.9 Hz, CHP), 41.80 (d,  $^1J_{C,P}$  = 139.6 Hz, CHP), 62.44 (d,  $^2J_{C,P}$  = 7.2 Hz,  $OCH_2CH_3$ ), 62.50 (d,  $^2J_{C,P}$  = 7.2 Hz,  $OCH_2CH_3$ ), 63.09 (d,  $^2J_{C,P}$  = 7.2 Hz,  $OCH_2CH_3$ ), 75.89 ( $OCH_2Ph$ ), 76.24 ( $OCH_2Ph$ ), 128.02 – 128.11 (=CH), 128.28 (=CH), 128.57 – 128.61 (=CH), 128.94 – 129.05 (=CH), 130.60 – 130.87 (=CH), 133.50 – 133.64 (=C), 133.70 – 134.02 (=C), 137.77 – 137.95 (=C), 148.21 (d,  $^3J_{C,P}$  = 17.0 Hz,  $HC=N$ ), 148.81 (d,  $^3J_{C,P}$  = 15.6 Hz,  $HC=N$ ) ppm. (most aromatic peaks are double due to coupling with phosphor or because of the presence of two isomers)

**Exact mass** (ESI-MS): calculated for  $C_{20}H_{26}ClNO_4P$   $[M+H]^+$ : 410.1288, found: 410.1284.

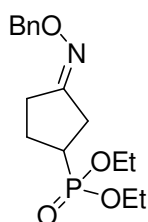
**Diethyl [(E)- and (Z)-3-(benzyloxy)imino-1-(3,4-dichlorophenyl)propyl]-phosphonate (3.10e).** Yield: 1.57 g (92%).



**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.17 (dt,  $J_{\text{H,P}} = 1.47$  Hz,  $J = 7.0$  Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.28 (dt,  $J_{\text{H,P}} = 3.2$  Hz,  $J = 7.0$  Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.72 – 3.05 (m, 2H, CHPC<sub>2</sub>H<sub>5</sub>), 3.17 – 3.32 (m, 1H, PCH), 3.81 – 3.90 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.93 – 3.98 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.99 – 4.10 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.97 (s, 1H, OCH<sub>2</sub>Ph), 5.08 (s, 1H, OCH<sub>2</sub>Ph), 6.55 (t,  $J = 5.3$  Hz, 1H, HC=N), 7.11 – 7.39 (m, 8H, arom. H) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>20</sub>H<sub>25</sub>Cl<sub>2</sub>NO<sub>4</sub>P [M + H]<sup>+</sup> 444.090, found 444.091.

**Diethyl {3-[(benzyloxy)imino]cyclopentyl}phosphonate (3.20).** Yield: 3.07 g (90%).



**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.30 (t,  $J = 7.0$  Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.31 (t,  $J = 7.0$  Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.80 – 2.89 (m, 7H, C<sub>5</sub>H<sub>7</sub>P), 4.04 – 4.15 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 5.06 (s, 2H, CH<sub>2</sub>Ph), 7.26 – 7.34 (m, 5H, arom. H) ppm.

**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.74 (d,  $^3J_{\text{C,P}} = 5.8$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 25.68 (d,  $J_{\text{C,P}} = 2.6$  Hz, CH<sub>2</sub>), 26.03 (d,  $J_{\text{C,P}} = 3.2$  Hz, CH<sub>2</sub>), 27.92 (d,  $J_{\text{C,P}} = 12.1$  Hz, CH<sub>2</sub>), 29.19 (d,  $J_{\text{C,P}} = 1.7$  Hz, CH<sub>2</sub>), 30.91 (d,  $J_{\text{C,P}} = 11.5$  Hz, CH<sub>2</sub>), 32.07 (d,  $J_{\text{C,P}} = 1.4$  Hz, CH<sub>2</sub>), 34.77 (d,  $J_{\text{C,P}} = 151.4$  Hz, CHP), 34.99 (d,  $J_{\text{C,P}} = 151.7$  Hz, CHP), 62.06 (m, OCH<sub>2</sub>CH<sub>3</sub>), 75.92 (OCH<sub>2</sub>Ph), 75.95 (OCH<sub>2</sub>Ph), 127.93 (arom. C), 127.95 (arom. C), 128.16 (arom. C), 128.18 (arom. C), 128.56 (arom. C), 128.55 (arom. C), 138.26 (arom. C), 138.33 (arom. C), 164.12 (d,  $^3J_{\text{C,P}} = 13.8$  Hz, C=N), 164.33 (d,  $^3J_{\text{C,P}} = 15.0$  Hz, C=N) ppm.

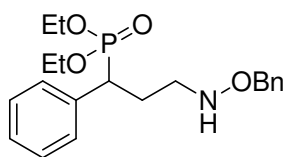
**<sup>31</sup>P NMR** (120 MHz, CDCl<sub>3</sub>): δ = 32.12, 32.36 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>16</sub>H<sub>25</sub>NO<sub>4</sub>P [M + H]<sup>+</sup> 326.1521, found 326.1523.

**General Procedure for the Reduction of the O-Benzoyloximes 3.10a-e to 3.11a-e and 3.20 to 3.21:** Sodium cyanoborohydride (12.95 mmol, 0.81 g) was added to a solution of O-benzoyloximes **3.10a-e** (2.59 mmol) in methanol (15 mL). Two drops of methyl orange indicator were added followed by dropwise addition of concentrated

hydrochloric acid, until the solution remained pink and milky for at least 0.5 h. The reaction mixture was stirred at room temperature for 3 – 16 h, and the solvent was removed under vacuum. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed until alkaline with 1 M potassium hydroxide solution, and the aqueous portion extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 mL). The combined organic extracts were dried with MgSO<sub>4</sub>, filtered and the solvent was removed. The residue was purified on a silica gel column and eluted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95:5 or *n*-hexane/ethyl acetate, 4:6. After concentration of the appropriate fractions, *O*-benzyloxyamines **3.11a-e** and **3.21** were obtained as clear oils.

**Diethyl [3-(benzyloxyamino)-1-phenylpropyl]phosphonate (3.11a).** Yield: 96%.

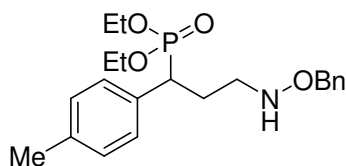


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.09 (dt, *J* = 7.0 Hz and *J*<sub>H,P</sub> = 0.6 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.27 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.14 – 2.30 (m, 1H, CH<sub>2</sub>CHP), 2.38 – 2.53 (m, 1H, CH<sub>2</sub>CHP), 2.81 – 2.90 (m, 1H, CH<sub>2</sub>N), 2.93 – 3.05 (m, 1H, CH<sub>2</sub>N), 3.25 (ddd, *J*<sub>H,P</sub> = 22.0 Hz, *J* = 4.7 Hz and *J* = 10.8 Hz, 1H, CHP), 3.64 – 3.79 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.85 – 3.97 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.99 – 4.14 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.81 (app s, 2H, PhCH<sub>2</sub>O), 7.22 – 7.37 (m, 10H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.48 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.64 (d, <sup>3</sup>*J*<sub>C,P</sub> = 6.0 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 26.99 (CH<sub>2</sub>CHP), 42.18 (d, <sup>1</sup>*J*<sub>C,P</sub> = 138.5 Hz, CHP), 49.42 (d, <sup>3</sup>*J*<sub>C,P</sub> = 15.3 Hz, NCH<sub>2</sub>), 61.28 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.02 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 76.64 (OCH<sub>2</sub>Ph), 127.61 (*J*<sub>C,P</sub> = 3.2 Hz, =CH), 128.49 (=CH), 128.71 (=CH), 128.87 (*J*<sub>C,P</sub> = 2.6 Hz, =CH), 128.93 (=CH), 129.48 (*J*<sub>C,P</sub> = 6.6 Hz, =CH), 135.65 (*J*<sub>C,P</sub> = 6.9 Hz, =C), 136.72 (=C) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>20</sub>H<sub>27</sub>NO<sub>4</sub>P [M-H]<sup>−</sup>: 376.1676, found: 376.1677.

**Diethyl [3-(benzyloxyamino)-1-*p*-tolylpropyl]phosphonate (3.11b).** Yield: 95%.



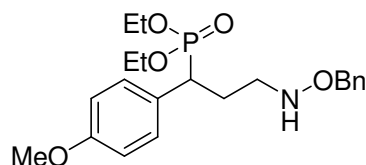
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.06 (m, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.25 (m, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.11 – 2.24 (m, 1H, CH<sub>2</sub>CHP), 2.25 (s, 3H, *p*-CH<sub>3</sub>), 2.38 – 2.87 (m, 1H, CH<sub>2</sub>CHP), 2.89 (m, 1H, CH<sub>2</sub>N), 2.96 (m, 1H, CH<sub>2</sub>N), 3.15 (ddd, *J*<sub>H,P</sub> = 22.4 Hz, *J* = 4.4 Hz and *J* = 11.4 Hz, 1H, CHP), 3.60 – 3.76 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.78 – 3.89 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.90 – 4.05 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.69 (app s, 2H, PhCH<sub>2</sub>O), 7.03 – 7.13 (m, 2H, arom. H), 7.26 – 7.31 (m, 7H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.52 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.67 (d, <sup>3</sup>*J*<sub>C,P</sub> = 6.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 21.34 (*p*-CH<sub>3</sub>), 27.60 (br d, CH<sub>2</sub>CHP), 41.71 (d, <sup>1</sup>*J*<sub>C,P</sub> = 139.0 Hz, CHP), 49.75 (d, <sup>3</sup>*J*<sub>C,P</sub> = 15.8 Hz, NCH<sub>2</sub>), 61.05 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.80 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 76.60 (OCH<sub>2</sub>Ph), 128.13 (=CH), 128.64 (d, =CH), 129.31 (=CH), 129.40 (=CH), 129.52 (=CH), 132.65 (d, *J*<sub>C,P</sub> = 6.9 Hz, =C), 137.07 (d, *J*<sub>C,P</sub> = 3.5 Hz, =C), 137.76 (=C) ppm.

**Mass** (ESI-MS): calculated for C<sub>21</sub>H<sub>31</sub>NO<sub>4</sub>P [M+H]<sup>+</sup>: 392.1991, found: 392.3.

### Diethyl [3-(benzyloxyamino)-1-(4-methoxyphenyl)propyl]phosphonate (3.11c).

Yield: 95%.



**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.10 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.27 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.03 – 2.19 (m, 1H, CH<sub>2</sub>CHP), 2.29 – 2.43 (m, 1H, CH<sub>2</sub>CHP), 2.71 – 2.80 (m, 1H, CH<sub>2</sub>N), 2.87 – 2.96 (m, 1H, CH<sub>2</sub>N), 3.18 (ddd, *J*<sub>H,P</sub> = 22.6 Hz, *J* = 3.8 Hz and *J* = 11.1 Hz, 1H, CHP), 3.65 – 3.80 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.78 (OCH<sub>3</sub>), 3.83 – 3.93 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.96 – 4.12 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.69 (app s, 2H, PhCH<sub>2</sub>O), 6.82 – 6.86 (m, 2H, arom. H), 7.21 – 7.34 (m, 7H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.54 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.67 (d, <sup>3</sup>*J*<sub>C,P</sub> = 6.3 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 27.77 (d, <sup>2</sup>*J*<sub>C,P</sub> = too small for detection, CH<sub>2</sub>CHP), 41.23 (d, <sup>1</sup>*J*<sub>C,P</sub> = 139.6 Hz, CHP), 49.77 (d, <sup>3</sup>*J*<sub>C,P</sub> = 15.8 Hz, NCH<sub>2</sub>), 55.54 (OCH<sub>3</sub>), 61.98 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.5 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.77 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 76.60 (OCH<sub>2</sub>Ph), 114.20 (d, *J*<sub>C,P</sub> = 0.03 Hz, =CH), 127.74 (d, *J*<sub>C,P</sub> = 7.2 Hz, =C), 128.07 (=CH), 128.60 (=CH), 129.26 (d, *J*<sub>C,P</sub> = 4.9 Hz, =CH), 131.50 (d, *J*<sub>C,P</sub> = 6.9 Hz, =CH), 137.93 (=C), 158.97 (d, *J*<sub>C,P</sub> = 3.2 Hz, =C) ppm.

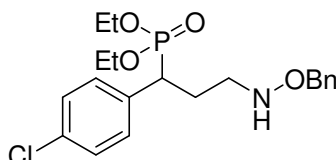
**<sup>31</sup>P-NMR** (120 MHz, CDCl<sub>3</sub>): δ = 30.20 ppm.



**Exact mass** (ESI-MS): calculated for  $C_{21}H_{31}NO_5P$   $[M+H]^+$ : 408.1940, found: 408.1932.

**Diethyl [3-(benzyloxyamino)-1-(4-chlorophenyl)propyl]phosphonate (3.11d).**

Yield: 93%.



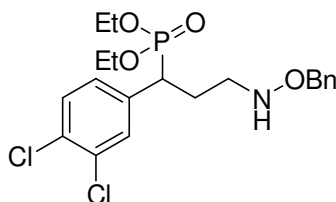
**$^1H$ -NMR** (300 MHz,  $CDCl_3$ ):  $\delta$  = 1.14 (t,  $J$  = 7.0 Hz, 3H,  $OCH_2CH_3$ ), 1.26 (dt,  $J$  = 7.0 Hz,  $J_{H,P}$  = 1.76 Hz, 3H,  $OCH_2CH_3$ ), 2.14 – 2.30 (m, 1H,  $CH_2CHP$ ), 2.42 – 2.59 (m, 1H,  $CH_2CHP$ ), 2.95 – 3.00 (m, 1H,  $CH_2N$ ), 3.01 – 3.12 (m, 1H,  $CH_2N$ ), 3.32 (ddd,  $J_{H,P}$  = 22.7 Hz,  $J_{HH}$  = 4.7 Hz and  $J$  = 10.3 Hz, 1H,  $CHP$ ), 3.73 – 3.87 (m, 1H,  $OCH_2CH_3$ ), 3.89 – 3.96 (m, 1H,  $OCH_2CH_3$ ), 3.97 – 4.12 (m, 2H,  $OCH_2CH_3$ ), 4.94 (s, 2H,  $PhCH_2O$ ), 7.18 – 7.38 (m, 9H, arom. H) ppm.

**$^{13}C$ -NMR** (75 MHz,  $CDCl_3$ ):  $\delta$  = 16.54 (app t,  $^3J_{C,P}$  = 6.3 Hz,  $OCH_2CH_3$ ), 25.75 (m,  $CH_2CHP$ ), 41.45 (d,  $^1J_{C,P}$  = 138.8 Hz,  $CHP$ ), 48.67 (d,  $^3J_{C,P}$  = 13.5 Hz,  $NCH_2$ ), 62.83 (d,  $^2J_{C,P}$  = 7.2 Hz,  $OCH_2CH_3$ ), 63.24 (d,  $^2J_{C,P}$  = 7.2 Hz,  $OCH_2CH_3$ ), 77.44 ( $OCH_2Ph$ ), 128.28 (=C), 128.70 (=CH), 128.88 (=CH), 129.16 (=CH), 129.41 (=CH), 130.72 (d,  $J_{C,P}$  = 6.6 Hz, =CH), 133.77 (d,  $J_{C,P}$  = 3.2 Hz, =C), 133.94 (d,  $J_{C,P}$  = 7.5 Hz, =C) ppm.

**Exact mass** (ESI-MS): calculated for  $C_{20}H_{28}ClNO_4P$   $[M+H]^+$ : 412.1445, found: 412.1312.

**Diethyl [3-(benzyloxyamino)-1-(3,4-dichlorophenyl)propyl]phosphonate (3.11e).**

Yield: 1.05 g (91%).



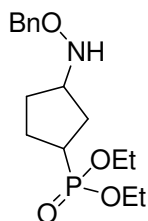
**$^1H$  NMR** (300 MHz,  $CDCl_3$ ):  $\delta$  = 1.16 (t,  $J$  = 7.0 Hz, 3H,  $OCH_2CH_3$ ), 1.28 (t,  $J$  = 7.0 Hz, 3H,  $OCH_2CH_3$ ), 1.97 – 2.13 (m, 1H,  $CH_2CHP$ ), 2.28 – 2.42 (m, 1H,  $CH_2CHP$ ), 2.64 – 2.74 (m, 1H,  $CH_2N$ ), 2.85 – 2.93 (m, 1H,  $CH_2N$ ), 3.20 (ddd,  $J_{H,P}$  = 22.6 Hz,  $J$  = 4.1, 11.1 Hz, 1H,  $CHP$ ), 3.77 – 3.89 (m, 1H,  $OCH_2CH_3$ ), 3.91 – 3.99 (m, 1H,

OCH<sub>2</sub>CH<sub>3</sub>), 3.99 – 4.13 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.61 – 4.70 (m, 2H, PhCH<sub>2</sub>O), 7.13 – 7.17 (m, 1H, arom. H), 7.26 – 7.40 (m, 7H, arom. H) ppm.

**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.56 (d, <sup>3</sup>J<sub>C,P</sub> = 7.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.64 (d, <sup>3</sup>J<sub>C,P</sub> = 8.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 27.73 (d, <sup>2</sup>J<sub>C,P</sub> = 2.9 Hz, CH<sub>2</sub>CHP), 41.35 (d, <sup>1</sup>J<sub>C,P</sub> = 139.6 Hz, CHP), 49.48 (d, <sup>3</sup>J<sub>C,P</sub> = 15.3 Hz, NCH<sub>2</sub>), 62.36 (d, <sup>2</sup>J<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.88 (d, <sup>2</sup>J<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 77.88 (OCH<sub>2</sub>Ph), 128.13 (arom. C), 128.61 (arom. C), 128.63 (arom. C), 128.87 (d, J<sub>C,P</sub> = 6.6 Hz, arom. C), 130.65 (d, J<sub>C,P</sub> = 2.6 Hz, arom. C), 131.42 (d, J<sub>C,P</sub> = 6.9 Hz, arom. C), 131.53 (arom. C), 132.74 (d, J<sub>C,P</sub> = 2.9 Hz, arom. C), 136.73 (d, J<sub>C,P</sub> = 6.9 Hz, arom. C), 137.88 (arom. C) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>20</sub>H<sub>27</sub>Cl<sub>2</sub>NO<sub>4</sub>P [M + H]<sup>+</sup> 446.1055, found 446.1060.

**Diethyl [3-(benzyloxyamino)cyclopentyl]phosphonate (3.21):** Yield: 1.83 g, mixture of *cis* and *trans* (80%).



**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.30 (t, J = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.30 (t, J = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.42 – 2.40 (m, 8H, C<sub>5</sub>H<sub>8</sub>P), 3.58 – 3.69 (m, 1H, NH), 4.03 – 4.15 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.69 (s, 1H, CH<sub>2</sub>Ph), 4.75 (s, 1H, CH<sub>2</sub>Ph), 7.26 – 7.56 (m, 5H, arom. H) ppm.

**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.76 (d, <sup>3</sup>J<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 25.27 (d, J<sub>C,P</sub> = 2.9 Hz, CH<sub>2</sub>), 25.72 (d, J<sub>C,P</sub> = 2.6 Hz, CH<sub>2</sub>), 30.18 (CH<sub>2</sub>), 30.34 (CH<sub>2</sub>), 31.54 (d, J<sub>C,P</sub> = 2.3 Hz, CH<sub>2</sub>), 31.67 (d, J<sub>C,P</sub> = 2.1 Hz, CH<sub>2</sub>), 33.73 (d, J<sub>C,P</sub> = 147.9 Hz, CHP), 34.55 (d, J<sub>C,P</sub> = 147.4 Hz, CHP), 61.65 – 62.13 (m, OCH<sub>2</sub>CH<sub>3</sub> and NCH), 77.59 (OCH<sub>2</sub>Ph), 76.95 (OCH<sub>2</sub>Ph), 128.11 (arom. C), 128.61 (arom. C), 128.67 (arom. C), 129.57 (arom. C), 130.42, 137.86 (arom. C) ppm.

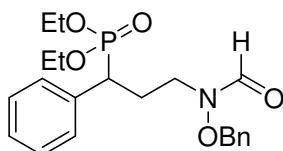
**Exact mass** (ESI-MS): calculated for C<sub>16</sub>H<sub>27</sub>NO<sub>4</sub>P [M + H]<sup>+</sup> 328.1678, found 328.1660.

## Formylation of Compounds 3.11 and 3.21

**Method A:** Formic acid (1 equiv.) and 2-mercaptothiazoline (1 equiv.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (0.5 M), cooled to 0 °C and DCC (1 equiv.) was added in one portion. After the reaction mixture was filtered and concentrated, the residue was purified by chromatography (CH<sub>2</sub>Cl<sub>2</sub>) to afford 2-thioxothiazolidine-3-carbaldehyde as a yellow solid.

### Diethyl {3-[*N*-(benzyloxy)formamido]-1-phenylpropyl}phosphonate (**3.12a**).

2-Thioxothiazolidine-3-carbaldehyde (1 equiv.) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and added to a solution of **11a** (1 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (0.1 M). The reaction mixture was stirred for 3 d. The reaction mixture was extracted with water, dried with MgSO<sub>4</sub> and concentrated in vacuo. The residue was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95:5) to yield **3.12a** in 89 % yield.



**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.07 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.26 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.17 (m, 1H, CH<sub>2</sub>CHP), 2.45 – 2.48 (m, 1H, CH<sub>2</sub>CHP), 3.06 (ddd, *J*<sub>H,P</sub> = 23.0 Hz, *J* = 4.1, 11.1 Hz, 1H, CHP), 3.39 (m, 1H, CH<sub>2</sub>N), 3.50 (m, 1H, CH<sub>2</sub>N), 3.62 – 3.75 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.81 – 3.91 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.97 – 4.10 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.74 and 4.94 (2 × br. s, 2H, PhCH<sub>2</sub>O), 7.26 – 7.35 (m, 10H, arom. H), 8.16 (br. s, 1H, HC=O) ppm.

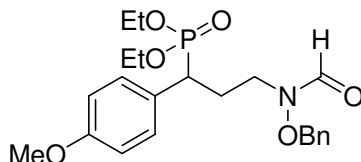
**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.40 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.58 (d, <sup>3</sup>*J*<sub>C,P</sub> = 6.0 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 27.42 (m, CH<sub>2</sub>CHP), 40 (d, <sup>1</sup>*J*<sub>C,P</sub> = 140 Hz, CHP), 43.11 (m, NCH<sub>2</sub>), 62.19 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.96 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 77.42 (OCH<sub>2</sub>Ph), 127.77 (arom. C), 128.91 (arom. C), 129.26 (arom. C), 129.47 (arom. C), 129.56 (arom. C), 129.63 (arom. C), 131.10 (arom. C), 135.09 (arom. C), 163.32 (m, HC=O) ppm.

**Mass** (ESI-MS): calculated for C<sub>21</sub>H<sub>29</sub>NO<sub>5</sub>P [M + H]<sup>+</sup> 406.1783, found 406.1.

**Method B:** To a three-neck flask containing a solution of formic acid (0.61 mmol, 30 μL) in CH<sub>2</sub>Cl<sub>2</sub> (0.6 mL) was added 1,1'-carbonyldiimidazole (0.64 mmol, 0.10 g). After 20 min, benzyloxyamines **3.11c,e** (0.61 mmol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) and then transferred to the three-neck flask. After 5 h, the mixture was partitioned between water (70 mL) and CH<sub>2</sub>Cl<sub>2</sub> (70 mL). The water layer was extracted twice with CH<sub>2</sub>Cl<sub>2</sub> (70 mL). The combined organic layers were dried with MgSO<sub>4</sub> and

concentrated in vacuo, and the residue was purified by flash chromatography (*n*-pentane/acetone, 6:4) to give **3.12c,e** as transparent oils.

**Diethyl {3-[*N*-(benzyloxy)formamido]-1-(4-methoxyphenyl)propyl}phosphonate (**3.12c**). Yield: 79%.**

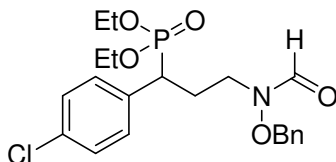


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.09 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.26 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.17 (m, 1H, CH<sub>2</sub>CHP), 2.41 – 2.49 (m, 1H, CH<sub>2</sub>CHP), 3.00 (ddd, *J*<sub>H,P</sub> = 22.9 Hz, *J* = 3.8 Hz and *J* = 11.4 Hz, 1H, CHP), 3.40 (m, 1H, CH<sub>2</sub>N), 3.55 (m, 1H, CH<sub>2</sub>N), 3.62 – 3.80 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.82 – 3.96 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.94 (OCH<sub>3</sub>), 3.97 – 4.11 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.74 and 4.94 (2 x br. s, 2H, PhCH<sub>2</sub>O), 6.84 – 7.36 (m, 9H, arom. H), 8.16 (br. s, 1H, HC=O) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.50 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.0 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.63 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 27.39 (m, CH<sub>2</sub>CHP), 40 (d, <sup>1</sup>*J*<sub>C,P</sub> = 140 Hz, CHP), 42.60 (m, NCH<sub>2</sub>), 55.47 (OCH<sub>3</sub>), 62.14 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.94 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 77.45 (OCH<sub>2</sub>Ph), 114.36 (arom. C), 126.80 (arom. C), 128.93 (arom. C), 129.32 (arom. C), 129.66 (arom. C), 130.50 (arom. C), 158.50 (arom. C), 159.21 (arom. C), 163.27 (m, HC=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>22</sub>H<sub>31</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 436.1889, found: 436.1888.

**Diethyl {3-[*N*-(benzyloxy)formamido]-1-(4-chlorophenyl)propyl}phosphonate (**3.12d**). Yield: 89%.**

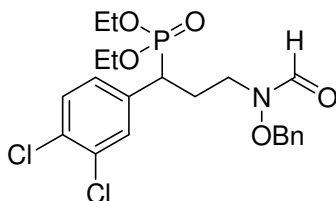


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.10 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.27 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.05 – 2.26 (m, 1H, CH<sub>2</sub>CHP), 2.38 – 2.53 (m, 1H, CH<sub>2</sub>CHP), 3.03 (ddd, *J*<sub>H,P</sub> = 23.2 Hz, *J* = 3.8 Hz and *J* = 11.1 Hz, 1H, CHP), 3.39 (m, 1H, CH<sub>2</sub>N), 3.47 (m, 1H, CH<sub>2</sub>N), 3.68 – 3.81 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.83 – 3.94 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.98 – 4.11 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.75 and 4.93 (2 x br. s, 2H, PhCH<sub>2</sub>O), 7.22 – 7.36 (m, 9H, arom. H), 8.16 (br. s, 1H, HC=O) ppm.

**$^{13}\text{C}$ -NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 16.48 (d,  $^3J_{\text{C,P}} = 5.8$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 16.61 (d,  $^3J_{\text{C,P}} = 5.8$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 27.37 (m,  $\text{CH}_2\text{CHP}$ ), 41.62 (d,  $^1J_{\text{CP}} = 139.6$  Hz,  $\text{CHP}$ ), 42.55 (m,  $\text{NCH}_2$ ), 62.37 (d,  $^2J_{\text{C,P}} = 7.2$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 63.04 (d,  $^2J_{\text{C,P}} = 6.9$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 77.45 ( $\text{OCH}_2\text{Ph}$ ), 128.97 (arom. C), 139.09 (arom. C), 129.38 (arom. C), 129.66 (arom. C), 130.77 (arom. C), 130.86 (arom. C), 133.68 (arom. C), 134.38 (arom. C), 163.27 (m,  $\text{HC=O}$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{21}\text{H}_{28}\text{ClNO}_5\text{P}$   $[\text{M}+\text{H}]^+$ : 440.1395, found: 440.1406.

**Diethyl {3-[*N*-(benzyloxy)formamido]-1-(3,4-dichlorophenyl)propyl}phosphonate (3.12e).** Yield: 245 mg (85%).

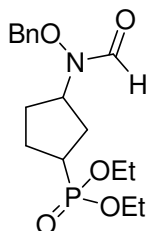


**$^1\text{H}$  NMR** (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.14 (t,  $J = 7.0$  Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.27 (t,  $J = 7.0$  Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 2.07 – 2.21 (m, 1H,  $\text{CH}_2\text{CHP}$ ), 2.37 – 2.51 (m, 1H,  $\text{CH}_2\text{CHP}$ ), 3.00 (ddd,  $J_{\text{H,P}} = 23.0$  Hz,  $J = 4.1, 11.4$  Hz, 1H,  $\text{CHP}$ ), 3.23 – 3.38 (m, 1H,  $\text{CH}_2\text{N}$ ), 3.44 – 3.46 (m, 1H,  $\text{CH}_2\text{N}$ ), 3.78 – 3.88 (m, 1H,  $\text{OCH}_2\text{CH}_3$ ), 3.89 – 3.99 (m, 1H,  $\text{OCH}_2\text{CH}_3$ ), 3.99 – 4.11 (m, 2H,  $\text{OCH}_2\text{CH}_3$ ), 4.75 and 4.91 (2  $\times$  br. s, 2H,  $\text{PhCH}_2\text{O}$ ), 7.12 – 7.40 (m, 8H, arom. H), 8.16 (br. s, 1H,  $\text{HC=O}$ ) ppm.

**$^{13}\text{C}$  NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 16.50 (d,  $^3J_{\text{C,P}} = 6.1$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 16.61 (d,  $^3J_{\text{C,P}} = 6.1$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 27.34 (m,  $\text{CH}_2\text{CHP}$ ), 41.46 (d,  $^1J_{\text{CP}} = 138.48$  Hz,  $\text{CHP}$ ), 42.50 (m,  $\text{NCH}_2$ ), 62.59 (d,  $^2J_{\text{C,P}} = 7.2$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 63.09 (d,  $^2J_{\text{C,P}} = 6.9$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 78.29 ( $\text{OCH}_2\text{Ph}$ ), 128.86 (arom. C), 128.95 (arom. C), 129.94 (arom. C), 129.69 (arom. C), 130.81 (arom. C), 131.24 (arom. C), 131.92 (arom. C), 132.93 (arom. C), 134.27 (arom. C), 135.87 (arom. C), 163.32 (m,  $\text{HC=O}$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{21}\text{H}_{27}\text{Cl}_2\text{NO}_5\text{P}$   $[\text{M} + \text{H}]^+$  474.1004, found 474.1000.

**Diethyl {3-[*N*-(benzyloxy)formamido]cyclopentyl}phosphonate (3.22).** Yield: 1.3 g, mixture of *cis* and *trans* (97%).



**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.23 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.64 – 2.39 (m, 7H, C<sub>5</sub>H<sub>7</sub>P), 3.98 – 4.09 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.4 (m, 1H, CHN), 4.90 (br. s, 2H, OCH<sub>2</sub>Ph), 7.30 – 7.32 (m, 5H, arom. H), 8.10 and 8.13 (2 × s, 1H, HC=O) ppm.

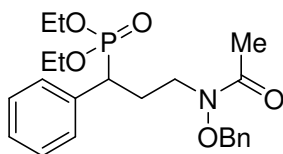
**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.76 (d, <sup>3</sup>J<sub>C,P</sub> = 5.8 Hz, 2 C, OCH<sub>2</sub>CH<sub>3</sub>), 24.40 (d, J<sub>C,P</sub> = 2.0 Hz, CH<sub>2</sub>), 25.60 (CH<sub>2</sub>), 29.98 (d, J<sub>C,P</sub> = 7.8 Hz, CH<sub>2</sub>), 33.31 (d, <sup>1</sup>J<sub>C,P</sub> = 150.6 Hz, CHP), 33.66 (d, <sup>1</sup>J<sub>C,P</sub> = 149.7 Hz, CHP), 57.73 (m, CHN), 59.03 (m, CHN), 62.01 (m, OCH<sub>2</sub>CH<sub>3</sub>), 79.50 (m, OCH<sub>2</sub>Ph), 128.95 – 129.63 (3 arom. C), 134.72 (arom. C), 165.10 (m, HC=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>17</sub>H<sub>27</sub>NO<sub>5</sub>P [M + H]<sup>+</sup> 356.1627, found 356.1629.

#### General method for synthesis of **3.13a-e** and **3.23**

A solution of benzyloxyamines **3.11a-e** (2.43 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (12 mL) containing (Et)<sub>3</sub>N (1.03 mL, 7.23 mmol) was cooled in an ice bath, followed by addition of acetylchloride (0.21 mL, 2.91 mmol). After 1.5 – 2 h the reaction was diluted with CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with water (100 mL). After drying of the organic layer with MgSO<sub>4</sub>, the solvent was removed *in vacuo* and the crude reaction mixture was chromatographed (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5 or *n*-hexane/ethyl acetate 6:4→1:1). Compounds **3.13a-e** were obtained as transparent oils.

**Diethyl {3-[*N*-(benzyloxy)acetamido]-1-phenylpropyl}phosphonate (3.13a).** Yield: 85%.



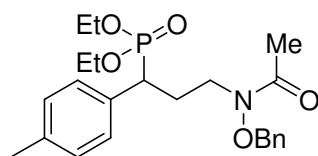
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.05 (t, *J* = 7.6 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.25 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.00 (s, 3H, CH<sub>3</sub>C=O), 2.18 – 2.34 (m, 1H, CH<sub>2</sub>CHP), 2.37 –

2.51 (m, 1H, CH<sub>2</sub>CHP), 3.06 (ddd,  $J_{\text{H,P}} = 23.0$  Hz,  $J = 4.1$  Hz and  $J = 11.4$  Hz, 1H, CHP), 3.39 – 3.49 (m, 1H, CH<sub>2</sub>N), 3.54 – 3.61 (m, 1H, CH<sub>2</sub>N), 3.63 – 3.74 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.79 – 3.95 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.95 – 4.07 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.67 (m, 2H, PhCH<sub>2</sub>O), 7.22 – 7.36 (m, 10H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>):  $\delta = 16.43$  (d,  $^3J_{\text{C,P}} = 5.8$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.60 (d,  $^3J_{\text{C,P}} = 6.0$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 20.67 (CH<sub>3</sub>C=O), 27.07 (CH<sub>2</sub>CHP), 42.34 (d,  $^1J_{\text{C,P}} = 137.9$  Hz, CHP), 44.01 (br m, NCH<sub>2</sub>), 62.24 (d,  $^2J_{\text{C,P}} = 7.2$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.98 (d,  $^2J_{\text{C,P}} = 7.2$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 76.54 (OCH<sub>2</sub>Ph), 127.62 (d,  $J_{\text{C,P}} = 3.2$  Hz, =CH), 128.82 (d,  $J_{\text{C,P}} = 2.9$  Hz, =CH), 128.89 (=CH), 129.16 (=CH), 129.41 (=CH), 129.52 (d,  $J_{\text{C,P}} = 6.6$  Hz, =CH), 134.49 (=C), 135.27 (d,  $J_{\text{C,P}} = 7.2$  Hz, =C), 172.56 (CH<sub>3</sub>C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>22</sub>H<sub>31</sub>NO<sub>5</sub>P [M+H]<sup>+</sup>: 420.1940, found: 420.1931.

**Diethyl {3-[N-(benzyloxy)acetamido]-1-*p*-tolylpropyl}phosphonate (3.13b).** Yield: 60%.

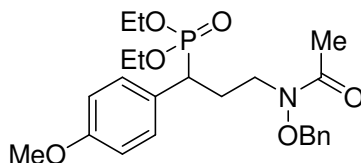


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>):  $\delta = 1.09$  (t,  $J = 7.0$  Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.25 (t,  $J = 7.0$  Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>C=O), 2.21 – 2.30 (m, 1H, CH<sub>2</sub>CHP), 2.32 (*p*-CH<sub>3</sub>), 2.39 – 2.46 (m, 1H, CH<sub>2</sub>CHP), 3.02 (ddd,  $J_{\text{H,P}} = 22.9$  Hz,  $J = 4.1$  Hz and  $J = 11.7$  Hz, 1H, CHP), 3.39 – 3.49 (m, 1H, CH<sub>2</sub>N), 3.60 (m, 1H, CH<sub>2</sub>N), 3.65 – 3.77 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.81 – 3.92 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.94 – 4.07 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.69 (m, 2H, PhCH<sub>2</sub>O), 7.11 – 7.38 (m, 9H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>):  $\delta = 16.47$  (d,  $^3J_{\text{C,P}} = 5.7$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.62 (d,  $^3J_{\text{C,P}} = 6.0$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 20.71 (CH<sub>3</sub>C=O), 21.32 (*p*-CH<sub>3</sub>), 27.05 (CH<sub>2</sub>CHP), 41.86 (d,  $^1J_{\text{C,P}} = 138.5$  Hz, CHP), 43.80 (br m, NCH<sub>2</sub>), 62.13 (d,  $^2J_{\text{C,P}} = 7.2$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.82 (d,  $^2J_{\text{C,P}} = 6.9$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 76.49 (OCH<sub>2</sub>Ph), 128.86 (=CH), 129.13 (=CH), 129.28 (=CH), 129.39 (d,  $J_{\text{C,P}} = 3.8$  Hz, =CH), 129.52 (d,  $J_{\text{C,P}} = 2.6$  Hz, =CH), 132.081 (d,  $J_{\text{C,P}} = 7.2$  Hz, =C), 134.52 (=C), 137.17 (d,  $J_{\text{C,P}} = 3.4$  Hz, =C), 172.50 (CH<sub>3</sub>C=O) ppm.

**Mass** (ESI-MS): calculated for C<sub>23</sub>H<sub>33</sub>NO<sub>5</sub>P [M+H]<sup>+</sup>: 434.2096, found: 434.3.

**Diethyl {3-[*N*-(benzyloxy)acetamido]-1-(4-methoxyphenyl)propyl}phosphonate (3.13c).** Yield: 93%.



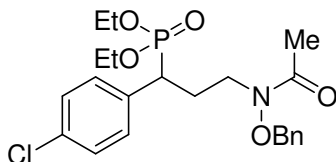
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.09 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.26 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>C=O), 2.14 – 2.30 (m, 1H, CH<sub>2</sub>CHP), 2.36 – 2.50 (m, 1H, CH<sub>2</sub>CHP), 3.01 (ddd, *J*<sub>H,P</sub> = 22.9 Hz, *J* = 3.5 Hz and *J* = 11.4 Hz, 1H, CHP), 3.40 – 3.49 (m, 1H, CH<sub>2</sub>N), 3.56 – 3.63 (m, 1H, CH<sub>2</sub>N), 3.65 – 3.76 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.80 (OCH<sub>3</sub>), 3.83 – 3.92 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.94 – 4.08 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.7 (m, 2H, PhCH<sub>2</sub>O), 6.84 – 7.38 (m, 9H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.52 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.5 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.64 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 20.65 (CH<sub>3</sub>C=O), 27.19 (CH<sub>2</sub>CHP), 41.44 (d, <sup>1</sup>*J*<sub>C,P</sub> = 138.8 Hz, CHP), 44.00 (br m, NCH<sub>2</sub>), 55.46 (OCH<sub>3</sub>), 62.11 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.85 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 76.54 (OCH<sub>2</sub>Ph), 114.24 (d, *J*<sub>C,P</sub> = 2.6 Hz, =CH), 127.13 (d, *J*<sub>C,P</sub> = 7.2 Hz, =C), 129.89 (=CH), 129.16 (=CH), 129.40 (=CH), 130.51 (d, *J*<sub>C,P</sub> = 6.6 Hz, =CH), 134.90 (=C), 159.07 (d, *J*<sub>C,P</sub> = 3.2 Hz, =C), 172.00 (CH<sub>3</sub>C=O) ppm.

**<sup>31</sup>P-NMR** (120 MHz, CDCl<sub>3</sub>): δ = 29.48 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>23</sub>H<sub>33</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 450.2046, found: 450.2043.

**Diethyl {3-[*N*-(benzyloxy)acetamido]-1-(4-chlorophenyl)propyl}phosphonate (3.13d).** Yield: 95%.



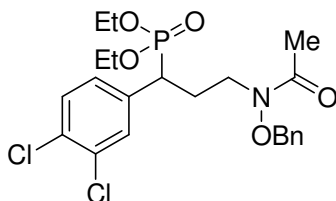
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.08 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.23 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.99 (s, 3H, CH<sub>3</sub>C=O), 2.09 – 2.27 (m, 1H, CH<sub>2</sub>CHP), 2.35 – 2.49 (m, 1H, CH<sub>2</sub>CHP), 3.01 (ddd, *J*<sub>H,P</sub> = 22.9 Hz, *J* = 3.8 Hz and *J* = 11.4 Hz, 1H, CHP), 3.37 – 3.46 (m, 1H, CH<sub>2</sub>N), 3.49 – 3.58 (m, 1H, CH<sub>2</sub>N), 3.66 – 3.79 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.81 – 3.91 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.93 – 4.06 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.67 (m, 2H, PhCH<sub>2</sub>O), 7.19 – 7.36 (m, 9H, arom. H) ppm.



**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.48 (d, <sup>3</sup>J<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.61 (d, <sup>3</sup>J<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 20.65 (CH<sub>3</sub>C=O), 27.02 (d, <sup>2</sup>J<sub>C,P</sub> = 2.3 Hz, CH<sub>2</sub>CHP), 41.74 (d, <sup>1</sup>J<sub>C,P</sub> = 139.1 Hz, CHP), 43.80 (br d, NCH<sub>2</sub>), 62.35 (d, <sup>2</sup>J<sub>C,P</sub> = 7.5 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.96 (d, <sup>2</sup>J<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 76.66 (OCH<sub>2</sub>Ph), 128.92 (=CH), 128.97 (=CH), 129.23 (=CH), 129.39 (=CH), 130.83 (d, J<sub>C,P</sub> = 6.9 Hz, =CH), 133.44 (d, J<sub>C,P</sub> = 4.0 Hz, =C), 134.03 (d, J<sub>C,P</sub> = 7.5 Hz, =C), 134.43 (=C), 172.53 (CH<sub>3</sub>C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>22</sub>H<sub>30</sub>ClNO<sub>5</sub>P [M+H]<sup>+</sup>: 454.1550, found: 454.1552.

**Diethyl {3-[N-(benzyloxy)acetamido]-1-(3,4-dichlorophenyl)propyl}phosphonate (3.13e).** Yield: 85%.

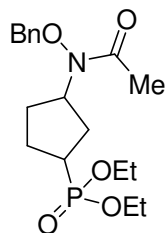


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.14 (t, J = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.26 (t, J = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>C=O), 2.08 – 2.26 (m, 1H, CH<sub>2</sub>CHP), 2.35 – 2.49 (m, 1H, CH<sub>2</sub>CHP), 3.00 (ddd, J<sub>H,P</sub> = 23.0 Hz, J = 3.8 Hz and J = 11.1 Hz, 1H, CHP), 3.27 – 3.59 (m, 2H, CH<sub>2</sub>N), 3.75 – 3.86 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.86 – 3.97 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.97 – 4.11 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.71 (m, 2H, PhCH<sub>2</sub>O), 7.12 – 7.43 (m, 8H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.48 (app t, <sup>3</sup>J<sub>C,P</sub> = 6.6 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 20.62 (CH<sub>3</sub>C=O), 27.01 (CH<sub>2</sub>CHP), 41.66 (d, <sup>1</sup>J<sub>C,P</sub> = 139.1 Hz, CHP), 44.07 (br m, NCH<sub>2</sub>), 62.52 (d, <sup>2</sup>J<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 62.98 (d, <sup>2</sup>J<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 76.80 (OCH<sub>2</sub>Ph), 128.91 (=CH), 129.33 (=CH), 129.60 (=CH), 130.40 (=CH), 130.67 (d, J<sub>C,P</sub> = 2.9 Hz, =CH), 131.30 (d, J<sub>C,P</sub> = 6.6 Hz, =CH), 131.69 (d, J<sub>C,P</sub> = 4.0 Hz, =C), 132.78 (d, J<sub>C,P</sub> = 3.2 Hz, =C), 134.39 (=C), 136.10 (d, J<sub>C,P</sub> = 7.2 Hz, =C), 172.54 (CH<sub>3</sub>C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>22</sub>H<sub>29</sub>Cl<sub>2</sub>NO<sub>5</sub>P [M+H]<sup>+</sup>: 488.1161, found: 488.1160.

**Diethyl {3-[*N*-(benzyloxy)acetamido]cyclopentyl}phosphonate (3.23).** Yield: 95% (mixture of *cis* and *trans*).



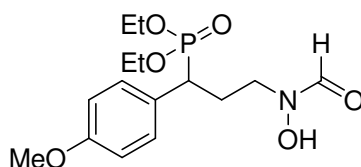
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.25 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.62 – 2.46 (m, 7H, C<sub>5</sub>H<sub>7</sub>P), 2.12 and 2.14 (2 x s, 3H, CH<sub>3</sub>C=O), 3.98 – 4.10 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.65 – 4.67 (m, 1H, CHN), 4.81 – 4.89 (m, 2H, OCH<sub>2</sub>Ph), 7.32 – 7.35 (m, 5H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.67 (d, <sup>3</sup>J<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 21.37 (CH<sub>3</sub>C=O), 21.65 (CH<sub>3</sub>C=O), 24.15 (d, J<sub>C,P</sub> = 3.2 Hz, CH<sub>2</sub>), 25.83 (d, J<sub>C,P</sub> = 3.5 Hz, CH<sub>2</sub>), 27.87 (s, CH<sub>2</sub>), 27.98 (s, CH<sub>2</sub>), 29.60 (m, CH<sub>2</sub>), 32.98 (d, J<sub>C,P</sub> = 149.4 Hz, CHP), 33.86 (d, J<sub>C,P</sub> = 148.3 Hz, CHP), 58.88 (d, J<sub>C,P</sub> = 18.13 Hz, CHN), 59.49 (d, J<sub>C,P</sub> = 8.64 Hz, CHN), 61.86 (m, OCH<sub>2</sub>CH<sub>3</sub>), 78.98 (OCH<sub>2</sub>Ph), 79.45 (OCH<sub>2</sub>Ph), 129.01 (two times three =CH signals), 134.60 (two =C signals), 174.23 (m, CH<sub>3</sub>C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>18</sub>H<sub>29</sub>NO<sub>5</sub>P [M+H]<sup>+</sup>: 370.1783, found: 370.1780.

**General Method for the Benzyl Deprotection of 3.12, 3.13, 3.22 and 3.23:** A solution of compounds **3.12** and **3.13** or **3.22** and **3.23** (0.9 mmol) in MeOH (8 mL) was hydrogenated at atmospheric pressure in the presence of Pd (10 wt.-% on activated carbon, 40 mg). After stirring for 5 h, the reaction mixture was filtered through a Celite pad. The solvent was removed under vacuum, and the crude mixture was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95:5).

**Diethyl [3-(*N*-hydroxyformamido)-1-(4-methoxyphenyl)propyl]phosphonate (3.14c).** Yield: 25%.



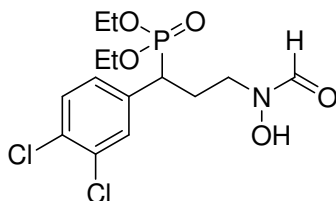
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.05 – 1.10 (m, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.17 – 1.23 (m, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.16 (m, 1H, CH<sub>2</sub>CHP), 2.53 (m, 1H, CH<sub>2</sub>CHP), 2.99 – 3.08 (m, 1H, CHP), 3.24 – 3.30 (m, 1H, CH<sub>2</sub>N), 3.40 – 3.62 (m, 1H, CH<sub>2</sub>N), 3.66 – 3.71 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.73 (OCH<sub>3</sub>), 3.81 – 3.86 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.91 – 3.99 (m, 2H,

OCH<sub>2</sub>CH<sub>3</sub>), 6.81 – 6.85 (m, 2H, *H<sub>m</sub>*), 7.17 – 7.19 (m, 2H, *H<sub>o</sub>*), 7.52 (br. s, 1H, HC=O), 8.31 (s, 1H, NOH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.05 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 16.15 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 26.40 (CH<sub>2</sub>CHP, major), 27.02 (CH<sub>2</sub>CHP, minor), 39.56 (d, <sup>1</sup>*J*<sub>C,P</sub> = 139.2 Hz, CHP, major), 40.66 (d, <sup>1</sup>*J*<sub>C,P</sub> = 137.2 Hz, CHP, minor), 44.56 (d, <sup>3</sup>*J*<sub>C,P</sub> = 16.6 Hz, NCH<sub>2</sub>, minor), 47.05 (d, <sup>3</sup>*J*<sub>C,P</sub> = 16.1 Hz, NCH<sub>2</sub>, major), 55.03 (OCH<sub>3</sub>), 62.07 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>, major), 62.25 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>, minor), 62.69 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.3 Hz, OCH<sub>2</sub>CH<sub>3</sub>, major), 62.83 (d, <sup>2</sup>*J*<sub>C,P</sub> = 5.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>, minor), 113.89 (=C<sub>m</sub>H, minor), 114.11 (=C<sub>m</sub>H, major), 125.94 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.3 Hz, =C<sub>i</sub>, major), 126.75 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.4 Hz, =C<sub>i</sub>, minor), 130.05 (=C<sub>o</sub>H), 158.77 (=C<sub>p</sub>, minor), 158.90 (=C<sub>p</sub>, major), 156.84 (C=O, major), 162.56 (C=O, minor) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>15</sub>H<sub>25</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 346.1420, found: 346.1427.

**Diethyl [1-(3,4-dichlorophenyl)-3-(*N*-hydroxyformamido)propyl]phosphonate (3.14e).** Yield: 157 mg (57%).



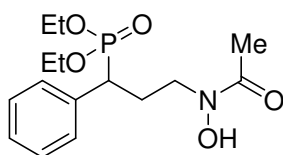
**<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.09 – 1.24 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 2.11 (m, 1H, CH<sub>2</sub>CHP), 2.46 (m, 1H, CH<sub>2</sub>CHP), 3.01 – 3.17 (m, 1H, CHP), 3.22 – 3.35 (m, 1H, CH<sub>2</sub>N), 3.45 – 3.56 (m, 1H, CH<sub>2</sub>N), 3.77 – 4.04 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 7.08 – 7.11 (m, 1H, arom. H), 7.32 – 7.37 (m, 2H, arom. H), 7.55 (br. s, 1H, HC=O), 8.24 (s, 1H, NOH) ppm.

**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.48 (app. t, <sup>3</sup>*J*<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 26.90 (CH<sub>2</sub>CHP, major), 27.08 (CH<sub>2</sub>CHP, minor), 40.09 (d, <sup>1</sup>*J*<sub>C,P</sub> = 139.3 Hz, CHP, major), 40.96 (d, <sup>1</sup>*J*<sub>C,P</sub> = 139.3 Hz, CHP, minor), 44.60 (d, <sup>3</sup>*J*<sub>C,P</sub> = 15.8 Hz, NCH<sub>2</sub>, minor), 47.45 (d, <sup>3</sup>*J*<sub>C,P</sub> = 15.0 Hz, NCH<sub>2</sub>, major), 62.84 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>, major), 63.01 (d, <sup>2</sup>*J*<sub>C,P</sub> = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub>, minor), 63.24 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.9 Hz, OCH<sub>2</sub>CH<sub>3</sub>, major), 63.32 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>, minor), 128.81 (d, *J*<sub>C,P</sub> = 6.3 Hz, arom. C, major), 128.95 (d, *J*<sub>C,P</sub> = 6.9 Hz, arom. C, minor), 130.69 (arom. C, minor), 130.91 (arom. C, major), 131.14 (d, *J*<sub>C,P</sub> = 6.9 Hz, arom. C, major), 131.24 (d, *J*<sub>C,P</sub> = 9.2 Hz, arom. C, minor), 131.74 (d, *J*<sub>C,P</sub> = 3.8 Hz, arom. C, minor), 131.98 (d, *J*<sub>C,P</sub> = 3.8 Hz,

arom. C, major), 132.70 (d,  $J_{C,P}$  = 2.6 Hz, arom. C, minor), 133.01 (d,  $J_{C,P}$  = 2.6 Hz, arom. C, major), 135.60 (d,  $J_{C,P}$  = 7.2 Hz, arom. C, major), 136.00 (d,  $J_{C,P}$  = 7.5 Hz, arom. C, minor), 157.37 (C=O, major), 163.03 (C=O, minor) ppm.

**Exact mass** (ESI-MS): calculated for  $C_{14}H_{21}Cl_2NO_5P$   $[M + H]^+$  384.0535, found 384.0530.

**Diethyl [3-(*N*-hydroxyacetamido)-1-phenylpropyl]phosphonate (3.15a).** Yield: 62%.

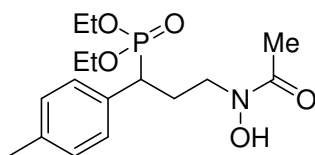


**$^1H$ -NMR** (300 MHz,  $CDCl_3$ ):  $\delta$  = 1.01 (m, 3H,  $OCH_2CH_3$ ), 1.19 (m, 3H,  $OCH_2CH_3$ ), 1.98 (s, 3H,  $CH_3C=O$ ), 2.20 (m, 1H,  $CH_2CHP$ ), 2.36 (m, 1H,  $CH_2CHP$ ), 3.04 (m, 1H,  $CHP$ ), 3.38 (m, 1H,  $CH_2N$ ), 3.62 (m, 1H,  $CH_2N$ ), 3.64 (m, 1H,  $OCH_2CH_3$ ), 3.78 (m, 1H,  $OCH_2CH_3$ ), 3.92 (m, 2H,  $OCH_2CH_3$ ), 7.23 (m, 5H, arom. H), 9.70 (br. s, 1H, NOH) ppm.

**$^{13}C$ -NMR** (75 MHz,  $CDCl_3$ ):  $\delta$  = 16.46 ( $OCH_2CH_3$ ), 20.65 ( $CH_3C=O$ ), 27.34 ( $CH_2CHP$ ), 42.11 (d,  $^1J_{C,P}$  = 137.7 Hz,  $CHP$ ), 46.34 (d,  $^3J_{C,P}$  = 17.0 Hz,  $NCH_2$ ), 62.57 ( $OCH_2CH_3$ ), 63.21 ( $OCH_2CH_3$ ), 127.66 (=CH), 128.80 (=CH), 129.38 (=CH), 135.43 (=C), 172.90 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for  $C_{15}H_{25}NO_5P$   $[M+H]^+$ : 330.1470, found: 330.1475.

**Diethyl [3-(*N*-hydroxyacetamido)-1-*p*-tolylpropyl]phosphonate (3.15b).** Yield: 20%.

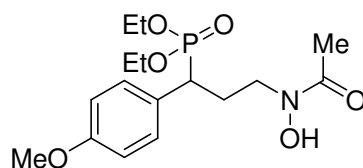


**$^1H$ -NMR** (300 MHz,  $CDCl_3$ ):  $\delta$  = 1.03 – 1.25 (m, 6H,  $OCH_2CH_3$ ), 2.10 (s, 3H,  $CH_3C=O$ ), 2.25 (s, 3H, *p*- $CH_3$ ), 2.20 (m, 1H,  $CH_2CHP$ ), 2.49 (m, 1H,  $CH_2CHP$ ), 2.95 – 3.07 (m, 1H,  $CHP$ ), 3.30 (m, 1H,  $CH_2N$ ), 3.47 (m, 1H,  $CH_2N$ ), 3.70 – 4.02 (m, 4H,  $OCH_2CH_3$ ), 7.04 – 7.12 (m, 4H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.50 (OCH<sub>2</sub>CH<sub>3</sub>), 20.66 (CH<sub>3</sub>C=O), 21.31 (*p*-CH<sub>3</sub>), 28.45 (CH<sub>2</sub>CHP), 41.68 (d, <sup>1</sup>J<sub>C,P</sub> = 135.9 Hz, CHP), 46.74 (d, <sup>3</sup>J<sub>C,P</sub> = 11.2 Hz, NCH<sub>2</sub>), 63.02 (d, <sup>2</sup>J<sub>C,P</sub> = 6.6 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.31 (d, <sup>2</sup>J<sub>C,P</sub> = 7.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 129.14 (=CH), 129.68 (=CH), 133.69 (=C), 137.34 (=C), 172.90 (C=O) ppm.

**Mass** (ESI-MS): calculated for C<sub>16</sub>H<sub>27</sub>NO<sub>5</sub>P [M+H]<sup>+</sup>: 344.1627, found: 344.2.

**Diethyl [3-(*N*-hydroxyacetamido)-1-(4-methoxyphenyl)propyl]phosphonate (3.15c).** Yield: 82%.

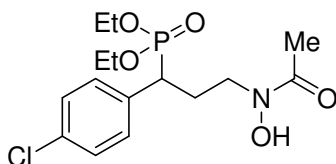


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.13 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.23 (t, *J* = 6.7 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.09 (s, 3H, CH<sub>3</sub>C=O), 2.17 (m, 1H, CH<sub>2</sub>CHP), 2.44 (m, 1H, CH<sub>2</sub>CHP), 3.04 (m, 1H, CHP), 3.36 (m, 1H, CH<sub>2</sub>N), 3.53 (m, 1H, CH<sub>2</sub>N), 3.77 (s, 3H, OCH<sub>3</sub>), 3.70 – 4.01 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 6.82 (2H, arom. H), 7.19 (2H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.52 (OCH<sub>2</sub>CH<sub>3</sub>), 20.70 (CH<sub>3</sub>C=O), 27.93 (CH<sub>2</sub>CHP), 41.22 (d, <sup>1</sup>J<sub>C,P</sub> = 138.2 Hz, CHP), 46.49 (d, <sup>3</sup>J<sub>C,P</sub> = 14.1 Hz, NCH<sub>2</sub>), 62.64 (OCH<sub>2</sub>CH<sub>3</sub>), 63.23 (OCH<sub>2</sub>CH<sub>3</sub>), 114.27 (=CH), 127.66 (=C), 130.34 (d, *J*<sub>C,P</sub> = 6.1 Hz, =CH), 159.08 (=C), 172.45 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>16</sub>H<sub>27</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 360.1576, found: 360.1573.

**Diethyl [1-(4-chlorophenyl)-3-(*N*-hydroxyacetamido)propyl]phosphonate (3.15d).** Yield: 70%.

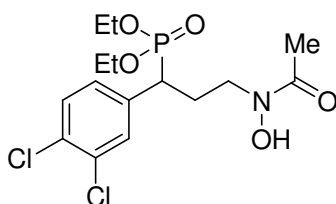


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 0.94 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.07 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.83 (s, 3H, CH<sub>3</sub>C=O), 2.00 (m, 1H, CH<sub>2</sub>CHP), 2.22 (m, 1H, CH<sub>2</sub>CHP), 2.88 – 2.98 (m, 1H, CHP), 3.23 (m, 1H, CH<sub>2</sub>N), 3.38 (m, 1H, CH<sub>2</sub>N), 3.55 – 3.64 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.67 – 3.75 (m, 1H, OCH<sub>2</sub>CH<sub>3</sub>), 3.77 – 3.84 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 7.09 (m, 4H, arom. H), 9.57 (br. s, 1H, NOH) ppm.

**$^{13}\text{C}$ -NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 16.29 (d,  $^3J_{\text{C,P}}$  = 5.8 Hz,  $\text{OCH}_2\text{CH}_3$ ), 16.40 (d,  $^3J_{\text{C,P}}$  = 6.0 Hz,  $\text{OCH}_2\text{CH}_3$ ), 20.35 ( $\text{CH}_3\text{C}=\text{O}$ ), 26.98 ( $\text{CH}_2\text{CHP}$ ), 41.27 (d,  $^1J_{\text{C,P}}$  = 138.8 Hz,  $\text{CHP}$ ), 45.99 (d,  $^3J_{\text{C,P}}$  = 17.3 Hz,  $\text{NCH}_2$ ), 62.55 (d,  $^2J_{\text{C,P}}$  = 7.5 Hz,  $\text{OCH}_2\text{CH}_3$ ), 63.04 (d,  $^2J_{\text{C,P}}$  = 7.2 Hz,  $\text{OCH}_2\text{CH}_3$ ), 128.75 ( $=\text{CH}$ ), 130.68 (d,  $J_{\text{C,P}}$  = 6.6 Hz,  $=\text{CH}$ ), 133.26 (d,  $J_{\text{C,P}}$  = 3.8 Hz,  $=\text{C}$ ), 133.88 (d,  $J_{\text{C,P}}$  = 7.5 Hz,  $=\text{C}$ ), 172.01 ( $\text{C}=\text{O}$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{15}\text{H}_{24}\text{ClNO}_5\text{P}$   $[\text{M}+\text{H}]^+$ : 364.1081, found: 364.1080.

**Diethyl [1-(3,4-dichlorophenyl)-3-(*N*-hydroxyacetamido)propyl]phosphonate (3.15e).** Yield: 62%.

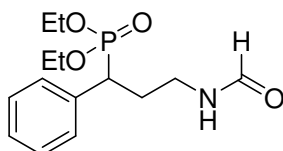


**$^1\text{H}$ -NMR** (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.23 (m, 6H,  $\text{OCH}_2\text{CH}_3$ ), 1.87 (m, 1H,  $\text{CH}_2\text{CHP}$ ), 2.11 (s, 3H,  $\text{CH}_3\text{C}=\text{O}$ ), 2.49 (m, 1H,  $\text{CH}_2\text{CHP}$ ), 3.07 (m, 1H,  $\text{CHP}$ ), 3.40 (m, 1H,  $\text{CH}_2\text{N}$ ), 3.59 (m, 1H,  $\text{CH}_2\text{N}$ ), 3.98 (m, 4H,  $\text{OCH}_2\text{CH}_3$ ), 7.14 (m, 1H, arom. H), 7.38 (m, 2H, arom. H), 9.45 (br. s, 1H, NOH) ppm.

**$^{13}\text{C}$ -NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 16.53 ( $\text{OCH}_2\text{CH}_3$ ), 20.80 ( $\text{CH}_3\text{C}=\text{O}$ ), 26.91 ( $\text{CH}_2\text{CHP}$ ), 41.37 (d,  $^1J_{\text{C,P}}$  = 139.4 Hz,  $\text{CHP}$ ), 46.34 ( $\text{NCH}_2$ ), 63.17 ( $\text{OCH}_2\text{CH}_3$ ), 63.47 ( $\text{OCH}_2\text{CH}_3$ ), 128.78 ( $=\text{CH}$ ), 130.71 ( $=\text{CH}$ ), 131.26 ( $=\text{CH}$ ), 131.81 ( $=\text{C}$ ), 132.816 ( $=\text{C}$ ), 136.87 ( $=\text{C}$ ), 172.73 ( $\text{C}=\text{O}$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{15}\text{H}_{22}\text{Cl}_2\text{NO}_5\text{P}$   $[\text{M}+\text{H}]^+$ : 398.0691, found: 398.0695.

**Diethyl [3-(formamido)-1-(phenyl)propyl]phosphonate (3.16a).** Yield: 76%.



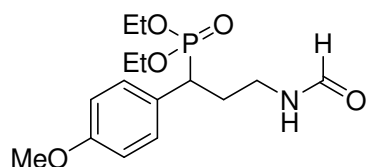
**$^1\text{H}$ -NMR** (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.11 (t,  $J$  = 6.7 Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.27 (t,  $J$  = 7.0 Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 2.06 – 2.23 (m, 1H,  $\text{CH}_2\text{CHP}$ ), 2.24 – 2.40 (m, 1H,  $\text{CH}_2\text{CHP}$ ), 3.08 (ddd,  $J_{\text{H,P}}$  = 22.9 Hz,  $J$  = 5.2 Hz and  $J$  = 9.9 Hz, 1H,  $\text{CH}_2\text{CHP}$ ), 3.16 – 3.37 (m, 2H,  $\text{NCH}_2$ ), 3.66 – 3.82 (m, 1H,  $\text{OCH}_2\text{CH}_3$ ), 3.84 – 3.97 (m, 1H,  $\text{OCH}_2\text{CH}_3$ ), 3.98 –

4.12 (m, 2H,  $\text{OCH}_2\text{CH}_3$ ), 5.90 (NH), 7.26 – 7.35 (m, 5H, arom. H), 8.11 (br. s, 1H,  $\text{HC}=\text{O}$ ) ppm.

**$^{13}\text{C}$ -NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 16.42 (d,  $^3J_{\text{C,P}} = 5.8$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 16.58 (d,  $^3J_{\text{C,P}} = 6.0$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 30.26 ( $\text{CH}_2\text{CHP}$ ), 36.75 (d,  $^3J_{\text{C,P}} = 14.7$  Hz,  $\text{NCH}_2$ ), 42.72 (d,  $^1J_{\text{C,P}} = 138.8$  Hz, CH), 62.30 (d,  $^2J_{\text{C,P}} = 7.2$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 63.03 (d,  $^2J_{\text{C,P}} = 7.2$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 127.75 (d,  $J_{\text{C,P}} = 2.8$  Hz, arom.  $=\text{C}_\text{m}\text{H}$ ), 128.95 (d,  $J_{\text{C,P}} = 2.2$  Hz, arom.  $=\text{C}_\text{i}$ ), 129.33 (d,  $J_{\text{C,P}} = 6.6$  Hz, arom.  $=\text{C}_\text{o}\text{H}$ ), 135.75 (d,  $J_{\text{C,P}} = 7.4$  Hz, arom.  $=\text{C}_\text{p}$ ), 161.35 ( $\text{C}=\text{O}$ ) ppm.

**Mass** (ESI-MS): calculated for  $\text{C}_{14}\text{H}_{23}\text{NO}_4\text{P}$   $[\text{M}+\text{H}]^+$ : 300.1324, found: 300.2.

**Diethyl [3-(formamido)-1-(4-methoxyphenyl)propyl]phosphonate (3.16c).** Yield: 27%.

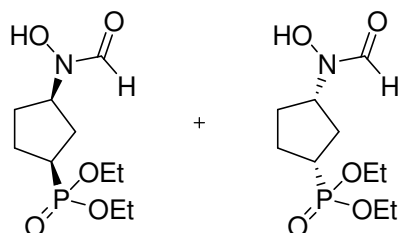


**$^1\text{H}$ -NMR** (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 0.998 (t,  $J = 7.1$  Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.15 (t,  $J = 6.9$  Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.94 – 2.04 (m, 1H,  $\text{CH}_2\text{CHP}$ ), 2.10 – 2.20 (m, 1H,  $\text{CH}_2\text{CHP}$ ), 2.95 (ddd,  $J_{\text{H,P}} = 22.7$  Hz,  $J = 4.1$  Hz and  $J = 11.0$  Hz, 1H,  $\text{CH}_2\text{CHP}$ ), 3.00 – 3.10 (m, 2H,  $\text{NCH}_2$ ), 3.58 – 3.65 (m, 1H,  $\text{OCH}_2\text{CH}_3$ ), 3.67 ( $\text{OCH}_3$ ), 3.73 – 3.81 (m, 1H,  $\text{OCH}_2\text{CH}_3$ ), 3.84 – 3.96 (m, 2H,  $\text{OCH}_2\text{CH}_3$ ), 6.72 – 6.77 (d,  $J_{\text{H,P}} = 8.5$  Hz, 2H, arom.  $\text{H}_\text{m}$ ), 7.04 (NH), 7.11 – 7.13 (d,  $J_{\text{H,P}} = 8.5$  Hz, 2H, arom.  $\text{H}_\text{o}$ ), 7.93 (br. s, 1H,  $\text{HC}=\text{O}$ ) ppm.

**$^{13}\text{C}$ -NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 15.89 (d,  $^3J_{\text{C,P}} = 4.8$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 16.03 (d,  $^3J_{\text{C,P}} = 5.0$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 29.45 ( $\text{CH}_2\text{CHP}$ ), 35.80 (d,  $^3J_{\text{C,P}} = 16.5$  Hz,  $\text{NCH}_2$ ), 40.89 (d,  $^1J_{\text{C,P}} = 116.2$  Hz,  $\text{CHP}$ ), 54.85 ( $\text{OCH}_3$ ), 61.68 (d,  $^2J_{\text{C,P}} = 7.8$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 62.35 (d,  $^2J_{\text{C,P}} = 6.7$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 113.93 (d,  $J_{\text{C,P}} = 2.0$  Hz,  $=\text{C}_\text{m}\text{H}$ ), 126.61 (d,  $J_{\text{C,P}} = 7.4$  Hz,  $=\text{C}_\text{i}$ ), 129.78 (d,  $J_{\text{C,P}} = 6.6$  Hz,  $=\text{C}_\text{o}\text{H}$ ), 158.58 (d,  $J_{\text{C,P}} = 2.8$  Hz,  $=\text{C}_\text{p}$ ), 161.25 ( $\text{C}=\text{O}$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{15}\text{H}_{25}\text{NO}_5\text{P}$   $[\text{M}+\text{H}]^+$ : 330.1430, found: 330.1454.

**Diethyl [3-(*N*-hydroxyformamido)cyclopentyl]phosphonate (*cis*-3.24).** Yield: 90 mg (19%).

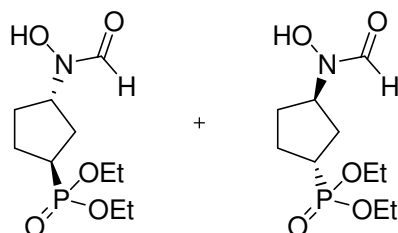


**$^1\text{H}$  NMR** (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.26 (m, 6H,  $\text{OCH}_2\text{CH}_3$ ), 1.75 – 2.42 (m, 7H,  $\text{C}_5\text{H}_7\text{P}$ ), 4.03 – 4.22 (m, 4H,  $\text{OCH}_2\text{CH}_3$ ), 4.83 (s, 1H,  $\text{CHNOH}$ ), 7.88 (s, 1H,  $\text{HC=O}$ ), 8.25 (s, 1H,  $\text{HC=O}$ ), 9.70 (s, 1H,  $\text{NOH}$ ) ppm.

**$^{13}\text{C}$  NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 16.65 (d,  $^3J_{\text{C,P}}$  = 5.8 Hz,  $\text{OCH}_2\text{CH}_3$ ), 26.15 ( $\text{CH}_2$ , major), 26.66 ( $\text{CH}_2$ , minor), 29.21 (d,  $J_{\text{C,P}}$  = 12.7 Hz,  $\text{CH}_2$ , minor), 29.81 (d,  $J_{\text{C,P}}$  = 11.5 Hz,  $\text{CH}_2$ , major), 29.81 ( $\text{CH}_2$ , minor), 30.44 ( $\text{CH}_2$ , major), 33.54 (d,  $^1J_{\text{C,P}}$  = 148.3 Hz,  $\text{CHP}$ , major), 35.51 (d,  $^1J_{\text{C,P}}$  = 148.9 Hz,  $\text{CHP}$ , minor), 55.08 (d,  $^3J_{\text{C,P}}$  = 12.7 Hz,  $\text{CHN}$ , major), 60.19 (d,  $^3J_{\text{C,P}}$  = 11.5 Hz,  $\text{CHN}$ , minor), 62.05 ( $\text{OCH}_2\text{CH}_3$ ), 62.15 ( $\text{OCH}_2\text{CH}_3$ ), 156.49 ( $\text{C=O}$ , major), 162.37 ( $\text{C=O}$ , minor) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{10}\text{H}_{21}\text{NO}_5\text{P}$   $[\text{M} + \text{H}]^+$  266.1158, found 266.1131.

**Diethyl [3-(*N*-hydroxyformamido)cyclopentyl]phosphonate (*trans*-3.24).** Yield: 170 mg (35%).



**$^1\text{H}$  NMR** (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.18 (t,  $J$  = 7.0 Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.18 (t,  $J$  = 6.8 Hz, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.75 – 2.08 (m, 7H,  $\text{C}_5\text{H}_7\text{P}$ ), 3.95 (m, 4H,  $\text{OCH}_2\text{CH}_3$ ), 4.68 (s, 1H,  $\text{CHNOH}$ ), 7.80 (s, 1H,  $\text{HC=O}$ , minor), 8.17 (s, 1H,  $\text{HC=O}$ , major), 9.78 (br. s, 1H,  $\text{NOH}$ ) ppm.

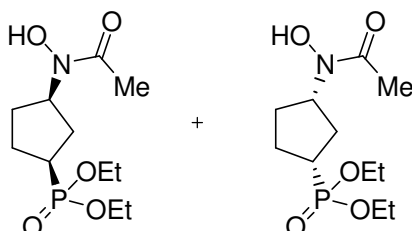
**$^{13}\text{C}$  NMR** (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 16.60 (d,  $^3J_{\text{C,P}}$  = 5.5 Hz,  $\text{OCH}_2\text{CH}_3$ ), 25.25 ( $\text{CH}_2$ ), 28.08 (d,  $J_{\text{C,P}}$  = 10.4 Hz,  $\text{CH}_2$ , major), 28.83 (d,  $J_{\text{C,P}}$  = 11.2 Hz,  $\text{CH}_2$ , minor), 29.4 (s,  $\text{CH}_2$ , major), 30.07 ( $\text{CH}_2$ , minor), 33.41 (d,  $^1J_{\text{C,P}}$  = 148.3 Hz,  $\text{CHP}$ , major), 34.22 (d,  $^1J_{\text{C,P}}$  = 150.0 Hz,  $\text{CHP}$ , minor), 55.44 (d,  $^3J_{\text{C,P}}$  = 15.8 Hz,  $\text{CHN}$ , major), 60.50 (d,  $^3J_{\text{C,P}}$



= 18.1 Hz, CHN, minor), 62.10 – 62.47 (m, 2C, OCH<sub>2</sub>CH<sub>3</sub>), 156.43 (C=O, minor), 162.48 (C=O, major) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>10</sub>H<sub>21</sub>NO<sub>5</sub>P [M + H]<sup>+</sup> 266.1158, found 266.1143.

**Diethyl [3-(*N*-hydroxyacetamido)cyclopentyl]phosphonate (*cis*-3.25).** Yield: 21%.

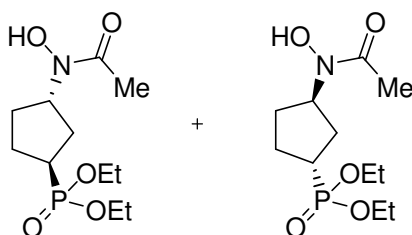


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.23 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 1.61 – 2.33 (m, 7H, C<sub>5</sub>H<sub>7</sub>P), 2.03 (m, 3H, CH<sub>3</sub>C=O), 3.99 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.96 (m, 1H, CHNOH), 9.60 (s, 1H, NOH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.67 (OCH<sub>2</sub>CH<sub>3</sub>), 21.02 (CH<sub>3</sub>C=O), 26.74 (CH<sub>2</sub>), 29.39 (CH<sub>2</sub>), 29.82 (CH<sub>2</sub>), 34.36 (d, <sup>1</sup>J<sub>C,P</sub> = 145.4 Hz, CHP), 55.52 (CHN), 62.12 (OCH<sub>2</sub>CH<sub>3</sub>), 171.95 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>11</sub>H<sub>23</sub>NO<sub>5</sub>P [M+H]<sup>+</sup>: 280.1314, found: 280.1311.

**Diethyl [3-(*N*-hydroxyacetamido)cyclopentyl]phosphonate (*trans*-3.25).** Yield: 46%.



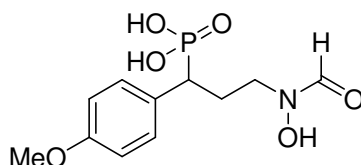
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.18 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.20 (t, *J* = 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.65 – 2.14 (m, 7H, C<sub>5</sub>H<sub>7</sub>P), 2.01 (m, 3H, CH<sub>3</sub>C=O), 3.96 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.85 (m, 1H, CHNOH), 9.53 (s, 1H, NOH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.58 (d, <sup>3</sup>J<sub>C,P</sub> = 4.3 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 20.95 (CH<sub>3</sub>C=O), 24.88 (CH<sub>2</sub>), 27.71 (d, *J*<sub>C,P</sub> = 9.2 Hz, CH<sub>2</sub>), 29.45 (CH<sub>2</sub>), 33.15 (d, <sup>1</sup>J<sub>C,P</sub> = 148.3 Hz, CHP), 56.11 (d, <sup>3</sup>J<sub>C,P</sub> = 15.8 Hz, CHN), 62.24 (m, OCH<sub>2</sub>CH<sub>3</sub>), 171.91 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for  $C_{11}H_{23}NO_5P$   $[M+H]^+$ : 280.1314, found: 280.1310.

**General Method for the Phosphonate Deprotection:** Esters **3.14**, **3.15**, **3.16**, **3.24** or **3.25** (0.84 mmol) were dissolved in  $CH_2Cl_2$  (10 mL) and treated dropwise with TMSBr (3.36 mmol, 0.50 g) under  $N_2$ . The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the volatile compounds were removed in vacuo to give the corresponding phosphonic acids in almost quantitative yield. All final compounds were purified using a preparative HPLC system on a C18 column (5  $\mu$ m, Phenomenex, Luna, 250  $\times$  21.2 mm) with a linear gradient of acetonitrile (0 $\rightarrow$ 100 %) in 5 mM  $NH_4OAc$  solution at a flow rate of 17.5 mL/min over 20 min. The compounds were detected by a UV-detector and ELSD-detector. The purity of all target compounds was assessed by analytical HPLC [5  $\mu$ m, Phenomenex, C18(2), 250  $\times$  4.6 mm] using the same gradient at a flow rate of 1 mL/min. All final compounds were obtained as hygroscopic powders after lyophilisation. All powders were white, except the five-membered cyclic analogues which were obtained as orange powders.

**[3-(*N*-Hydroxyformamido)-1-(4-methoxyphenyl)propyl]phosphonic acid (3.1c).**



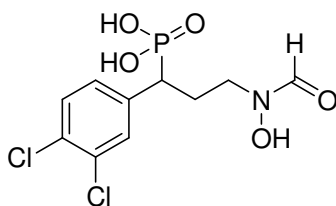
**$^1H$  NMR** (300 MHz,  $D_2O$ ):  $\delta$  = 1.97 – 2.12 (m, 1H,  $\beta$ -CH), 2.24 – 2.38 (m, 1H,  $\beta$ -CH), 2.74 (ddd,  $J_{H,P}$  = 21.9 Hz,  $J$  = 3.2 Hz and  $J$  = 15.8 Hz, 1H,  $\alpha$ -CH), 3.13 – 3.47 (m, 2H,  $\gamma$ -CH<sub>2</sub>), 3.71 (s, 3H, OCH<sub>3</sub>), 6.83 – 6.87 (m, 2H, arom. H), 7.12 – 7.15 (m, 2H, arom. H), 7.38 and 8.08 (2  $\times$  s, 1H, major and minor HC=O) ppm.

**$^{13}C$  NMR** (75 MHz,  $D_2O$ ):  $\delta$  = 26.59 (s,  $\beta$ -CH<sub>2</sub>), 42.37 (d,  $\alpha$ -CH,  $^1J_{C,P}$  = 131.3 Hz), 48.87 (d,  $\gamma$ -CH<sub>2</sub>,  $^3J_{C,P}$  = 17.3 Hz), 55.55 (s, OCH<sub>3</sub>), 114.28 (d,  $J_{C,P}$  = 2.3 Hz, =CH), 130.33 (d,  $J_{C,P}$  = 6.1 Hz, =CH), 130.53 (d,  $J_{C,P}$  = 7.2 Hz, =C), 157.74 (d,  $J_{C,P}$  = 2.9 Hz, =C), 159.74 and 163.70 (2  $\times$  s, major and minor C=O) ppm.

**$^{31}P$  NMR** (121 MHz,  $D_2O$ ):  $\delta$  = 23.18 and 23.47 ppm. (major and minor isomer)

**Exact mass** (ESI-MS): calculated for  $C_{11}H_{15}NO_6P$   $[M-H]^-$ : 288.0636, found: 288.0630.

**[1-(3,4-Dichlorophenyl)-3-(*N*-hydroxyformamido)propyl]phosphonic acid (3.1e).**



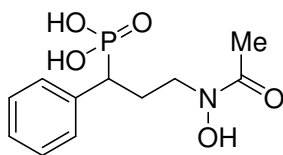
**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 1.93 – 2.15 (m, 1H,  $\beta$ -CH), 2.24 – 2.38 (m, 1H,  $\beta$ -CH), 2.73 – 2.87 (m, 1H,  $\alpha$ -CH), 3.17 – 3.47 (m, 2H,  $\gamma$ -CH<sub>2</sub>), 7.07 – 7.12 (m, 1H, arom. H), 7.33 – 7.39 (m, 2H, arom. H), 7.44 and 8.07 (2  $\times$  s, 1 H, HC=O) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O):  $\delta$  = 26.49 (s,  $\beta$ -CH<sub>2</sub>), 42.82 (d,  $^1J_{C,P}$  = 129.6 Hz,  $\alpha$ -CH), 48.86 (d,  $^3J_{C,P}$  = 17.0 Hz,  $\gamma$ -CH<sub>2</sub>), 128.92 (d,  $J_{C,P}$  = 5.8 Hz, arom. C), 130.04 (d,  $J_{C,P}$  = 3.8 Hz, arom. C), 130.55 (d,  $J_{C,P}$  = 2.6 Hz, arom. C), 130.73 (d,  $J_{C,P}$  = 6.0 Hz, arom. C), 131.88 (d,  $J_{C,P}$  = 3.2 Hz, arom. C), 138.80 (d,  $J_{C,P}$  = 7.2 Hz, arom. C), 159.70 (C=O, major) and 163.76 (C=O, minor) ppm.

**<sup>31</sup>P NMR** (121 MHz, D<sub>2</sub>O):  $\delta$  = 21.46, 21.78 (major and minor isomers) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>10</sub>H<sub>11</sub>Cl<sub>2</sub>NO<sub>5</sub>P [M-H]<sup>-</sup> 325.9751, found 325.9745.

**[3-(*N*-Hydroxyacetamido)-1-phenylpropyl]phosphonic acid (3.2a).**

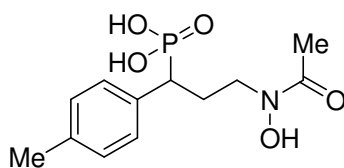


**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 1.57 and 1.90 (2  $\times$  s, 3H, minor and major CH<sub>3</sub>), 2.04 – 2.14 (m, 1H,  $\beta$ -CH), 2.19 – 2.27 (m, 1H,  $\beta$ -CH), 2.70 – 2.87 (m, 1H,  $\alpha$ -CH), 3.21 – 3.30 (m, 1H,  $\gamma$ -CH), 3.38 – 3.53 (m, 1H,  $\gamma$ -CH), 7.16 – 7.27 (m, 5H, arom. H) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O):  $\delta$  = 19.35 (s, CH<sub>3</sub>), 26.99 (s,  $\beta$ -CH<sub>2</sub>), 44.06 (d,  $^1J_{C,P}$  = 129.3 Hz,  $\alpha$ -CH), 46.36 (d,  $^3J_{C,P}$  = 17.3 Hz,  $\gamma$ -CH<sub>2</sub>), 126.59 (d, =CH), 128.51 (d,  $J_{C,P}$  = 2.3 Hz, =CH), 129.29 (d,  $J_{C,P}$  = 5.8 Hz, =CH), 138.93 (m,  $J_{C,P}$  = 7.2 Hz, =C), 173.72 (s, C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>11</sub>H<sub>15</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 272.0687, found: 272.0684.

**[3-(*N*-Hydroxyacetamido)-1-*p*-tolylpropyl]phosphonic acid (3.2b).**

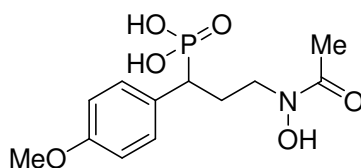


**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 1.48 and 1.80 (2 × s, 3H, minor and major CH<sub>3</sub>), 1.84 – 2.04 (m, 1H,  $\beta$ -CH), 2.10 (s, 3H, *p*-CH<sub>3</sub>), 2.15 – 2.32 (m, 1H,  $\beta$ -CH), 2.58 – 2.70 (m, 1H,  $\alpha$ -CH), 3.12 – 3.20 (m, 1H,  $\gamma$ -CH), 3.24 – 3.42 (m, 1H,  $\gamma$ -CH), 6.99 – 7.02 (m, 4H, arom. H) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O):  $\delta$  = 19.25 (s, CH<sub>3</sub>), 20.15 (s, *p*-CH<sub>3</sub>), 26.87 (s,  $\beta$ -CH<sub>2</sub>), 43.50 (d, <sup>1</sup>*J*<sub>C,P</sub> = 129.8 Hz,  $\alpha$ -CH), 46.30 (d, <sup>3</sup>*J*<sub>C,P</sub> = 17.6 Hz,  $\gamma$ -CH<sub>2</sub>), 128.98 (d, *J*<sub>C,P</sub> = 2.3 Hz, =CH), 129.12 (d, *J*<sub>C,P</sub> = 6.0 Hz, =CH), 135.50 (d, *J*<sub>C,P</sub> = 6.9 Hz, =C), 136.54 (d, *J*<sub>C,P</sub> = 3.2 Hz, =C), 173.60 (s, C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>12</sub>H<sub>17</sub>NO<sub>5</sub>P [M-H]<sup>−</sup>: 286.0843, found: 286.0839.

**[3-(*N*-Hydroxyacetamido)-1-(4-methoxyphenyl)propyl]phosphonic acid (3.2c).**



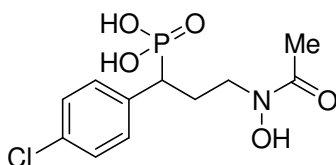
**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 1.55 and 1.88 (2 × s, 3H, minor and major CH<sub>3</sub>), 1.98 – 2.11 (m, 1H,  $\beta$ -CH), 2.15 – 2.35 (m, 1H,  $\beta$ -CH), 2.67 – 2.84 (m, 1H,  $\alpha$ -CH), 3.23 – 3.34 (m, 1H,  $\gamma$ -CH), 3.35 – 3.50 (m, 1H,  $\gamma$ -CH), 3.70 (s, 1H, OCH<sub>3</sub>), 6.82 – 6.88 (m, 2H, arom. H), 7.11 – 7.17 (m, 2H, arom. H) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O):  $\delta$  = 19.33 (s, CH<sub>3</sub>), 26.89 (s,  $\beta$ -CH<sub>2</sub>), 43.04 (d, <sup>1</sup>*J*<sub>C,P</sub> = 131.3 Hz,  $\alpha$ -CH), 46.35 (d, <sup>3</sup>*J*<sub>C,P</sub> = 17.3 Hz,  $\gamma$ -CH<sub>2</sub>), 55.55 (s, OCH<sub>3</sub>), 114.16 (d, =CH), 130.32 (d, *J*<sub>C,P</sub> = 6.1 Hz, =CH), 131.10 (d, *J*<sub>C,P</sub> = 7.2 Hz, =C), 157.61 (m, =C), 173.70 (s, C=O) ppm.

**<sup>31</sup>P NMR** (121 MHz, D<sub>2</sub>O):  $\delta$  = 23.32 and 23.67 ppm. (minor and major isomer)

**Exact mass** (ESI-MS): calculated for C<sub>12</sub>H<sub>17</sub>NO<sub>6</sub>P [M-H]<sup>−</sup>: 302.0792, found: 302.0794.

**[1-(4-Chlorophenyl)-3-(*N*-hydroxyacetamido)propyl]phosphonic acid (3.2d).**

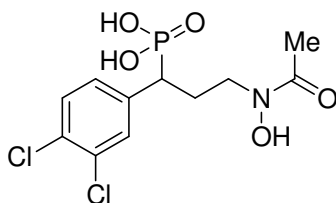


**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 1.58 and 1.87 (2 × s, 3H, minor and major CH<sub>3</sub>), 1.95 – 2.14 (m, 1H,  $\beta$ -CH), 2.17 – 2.36 (m, 1H,  $\beta$ -CH), 2.72 – 2.84 (m, 1H,  $\alpha$ -CH), 3.22 – 3.32 (m, 1H,  $\gamma$ -CH), 3.35 – 3.50 (m, 1H,  $\gamma$ -CH), 7.13 – 7.20 (m, 2H, arom. H), 7.20 – 7.28 (m, 2H, arom. H) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O):  $\delta$  = 19.28 (s, CH<sub>3</sub>), 26.77 (s,  $\beta$ -CH<sub>2</sub>), 43.46 (d, <sup>1</sup>*J*<sub>C,P</sub> = 129.6 Hz,  $\alpha$ -CH), 46.23 (d, <sup>3</sup>*J*<sub>C,P</sub> = 17.6 Hz,  $\gamma$ -CH<sub>2</sub>), 128.40 (d, =CH), 130.65 (d, *J*<sub>C,P</sub> = 5.8 Hz, =CH), 131.77 (d, =C), 137.24 (d, *J*<sub>C,P</sub> = 7.2 Hz, =C), 173.70 (s, C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>11</sub>H<sub>14</sub>ClNO<sub>5</sub>P [M-H]<sup>-</sup>: 306.0297, found: 306.0293.

**[1-(3,4-Dichlorophenyl)-3-(*N*-hydroxyacetamido)propyl]phosphonic acid (3.2e).**



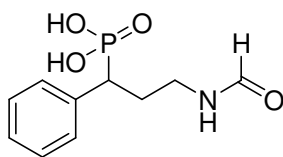
**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 1.61 and 1.86 (2 × s, 3H, minor and major CH<sub>3</sub>), 1.97 – 2.12 (m, 1H,  $\beta$ -CH), 2.17 – 2.34 (m, 1H,  $\beta$ -CH), 2.78 (ddd, *J*<sub>H,P</sub> = 21.8 Hz, *J* = 2.9 Hz and *J* = 12.3 Hz, 1H,  $\alpha$ -CH), 3.25 – 3.34 (m, 1H,  $\gamma$ -CH), 3.36 – 3.49 (m, 1H,  $\gamma$ -CH), 7.06 – 7.13 (m, 1H, arom. H), 7.32 – 7.40 (m, 2H, arom. H) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O):  $\delta$  = 19.23 (s, CH<sub>3</sub>), 26.68 (s,  $\beta$ -CH<sub>2</sub>), 43.36 (d, <sup>1</sup>*J*<sub>C,P</sub> = 129.3 Hz,  $\alpha$ -CH), 48.86 (d, <sup>3</sup>*J*<sub>C,P</sub> = 17.0 Hz,  $\gamma$ -CH<sub>2</sub>), 128.93 (d, *J*<sub>C,P</sub> = 5.8 Hz, =CH), 129.78 (d, =C), 130.26 (d, =CH), 130.93 (d, *J*<sub>C,P</sub> = 6.3 Hz, =CH), 131.51 (d, =C), 139.24 (d, *J*<sub>C,P</sub> = 7.2 Hz, =C), 173.70 (s, C=O) ppm.

**<sup>31</sup>P NMR** (121 MHz, D<sub>2</sub>O):  $\delta$  = 21.51 and 21.90 ppm. (minor and major isomer)

**Exact mass** (ESI-MS): calculated for C<sub>11</sub>H<sub>13</sub>Cl<sub>2</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 339.9907, found: 339.9901.

**[3-(Formamido)-1-(phenyl)propyl]phosphonic acid (3.17a).**

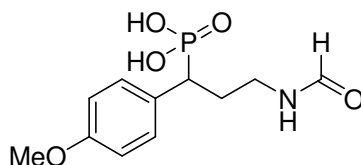


**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 2.05 – 2.29 (m, 2H,  $\beta$ -CH), 2.88 – 3.00 (m, 1H,  $\alpha$ -CH), 3.02 – 3.20 (m, 2H,  $\gamma$ -CH<sub>2</sub>), 7.26 – 7.39 (m, 5H, arom. H), 7.90 (s, 1H, HC=O) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O):  $\delta$  = 29.29 (s,  $\beta$ -CH<sub>2</sub>), 36.53 (d,  $^3J_{C,P}$  = 17.0 Hz,  $\gamma$ -CH<sub>2</sub>), 43.99 (d,  $^1J_{C,P}$  = 129.8 Hz,  $\alpha$ -CH), 126.63 (d,  $J_{C,P}$  = 2.9 Hz, =CH), 128.53 (d,  $J_{C,P}$  = 2.3 Hz, =CH), 129.22 (d,  $J_{C,P}$  = 6.1 Hz, =CH), 138.70 (d,  $J_{C,P}$  = 6.6 Hz, =C), 164.11 (s, C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>10</sub>H<sub>13</sub>NO<sub>4</sub>P [M-H]<sup>-</sup>: 242.0581, found: 242.0590.

**[3-(Formamido)-1-(4-methoxyphenyl)propyl]phosphonic acid (3.17c).**



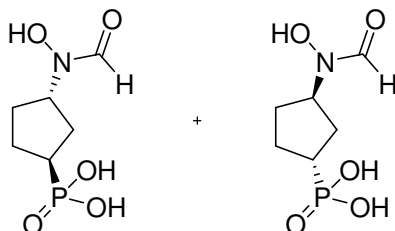
**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 1.85 – 1.99 (m, 1H,  $\beta$ -CH), 1.99 – 2.12 (m, 1H,  $\beta$ -CH), 2.69 – 2.81 (m, 1H,  $\alpha$ -CH), 2.82 – 3.04 (m, 2H,  $\gamma$ -CH<sub>2</sub>), 3.65 (s, 3H, OCH<sub>3</sub>), 6.78 – 6.83 (m, 2H, arom. H), 7.08 – 7.12 (m, 2H, arom. H), 7.74 (s, 1H, HC=O) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O):  $\delta$  = 29.38 (s,  $\beta$ -CH<sub>2</sub>), 36.55 (d,  $\gamma$ -CH<sub>2</sub>,  $^3J_{C,P}$  = 17.0 Hz), 43.00 (d,  $\alpha$ -CH,  $^1J_{C,P}$  = 131.3 Hz), 55.52 (s, OCH<sub>3</sub>), 114.06 (d,  $J_{C,P}$  = 2.3 Hz, =CH), 130.30 (d,  $J_{C,P}$  = 6.0 Hz, =CH), 131.11 (d,  $J_{C,P}$  = 7.2 Hz, =C), 157.61 (d,  $J_{C,P}$  = 2.9 Hz, =C), 164.13 (s, C=O) ppm.

**<sup>31</sup>P NMR** (121 MHz, D<sub>2</sub>O):  $\delta$  = 23.69 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>11</sub>H<sub>15</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 272.0687, found: 272.0693.

**[(1*R*,3*R*)-3-(*N*-Hydroxyformamido)cyclopentyl]phosphonic acid and [(1*S*,3*S*)-3-(*N*-hydroxyformamido)cyclopentyl]phosphonic acid (*trans*-3.3).**



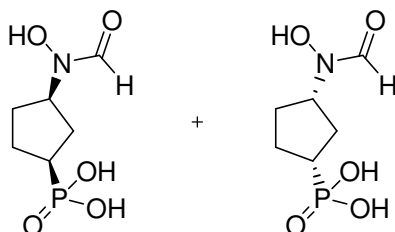
**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O): δ = 1.73 – 2.04 (m, 7H, α-CH and CH<sub>2</sub>), 4.13 (br. s, 1H, NCH), 7.84, 8.07 (2 × s, 1H, major and minor HC=O) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O): δ = 25.55 (d, *J*<sub>C,P</sub> = 1.2 Hz, CH<sub>2</sub>), 28.56 (d, *J*<sub>C,P</sub> = 10.4 Hz, CH<sub>2</sub>), 31.10 (s, CH<sub>2</sub>), 35.94 (d, <sup>1</sup>*J*<sub>C,P</sub> = 141.7 Hz, α-CH), 61.46 (d, *J*<sub>C,P</sub> = 17.3 Hz, NCH), 159.23 (C=O) ppm.

**<sup>31</sup>P NMR** (121 MHz, D<sub>2</sub>O): δ = 27.71, 27.91 (major and minor isomers) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>6</sub>H<sub>11</sub>NO<sub>5</sub>P [M-H]<sup>-</sup> 208.0374, found 208.0366.

**[(1*R*,3*S*)-3-(*N*-Hydroxyformamido)cyclopentyl]phosphonic acid and [(1*S*,3*R*)-3-(*N*-hydroxyformamido)cyclopentyl]phosphonic acid (*cis*-3.3).**



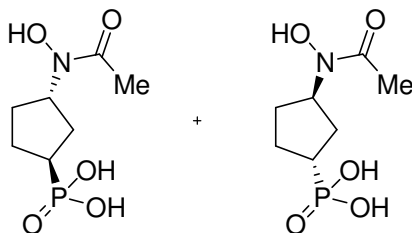
**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O): δ = 1.49 – 2.17 (m, 7H, α-CH and CH<sub>2</sub>), 4.21 (br. m, 1H, NCH), 7.88, 8.09 (2 × s, 1H, major and minor HC=O) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O): δ = 26.73 (d, *J*<sub>C,P</sub> = 1.1 Hz, CH<sub>2</sub>), 29.89 (d, *J*<sub>C,P</sub> = 11.2 Hz, CH<sub>2</sub>), 30.76 (CH<sub>2</sub>), 36.47 (d, <sup>1</sup>*J*<sub>C,P</sub> = 141.5 Hz, α-CH), 61.36 (d, *J*<sub>C,P</sub> = 10.9 Hz, NCH), 159.11 (C=O) ppm.

**<sup>31</sup>P NMR** (121 MHz, D<sub>2</sub>O): δ = 27.74 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>6</sub>H<sub>11</sub>NO<sub>5</sub>P [M-H]<sup>-</sup> 208.0374, found 208.0378.

**[(1*R*,3*R*)-3-(*N*-Hydroxyacetamido)cyclopentyl]phosphonic acid and [(1*S*,3*S*)-3-(*N*-hydroxyacetamido)cyclopentyl]phosphonic acid (*trans*-3.4).**



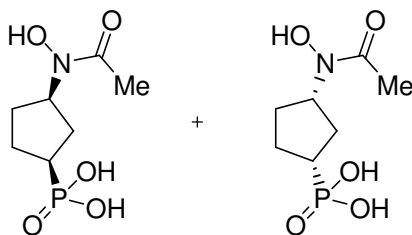
**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O): δ = 1.67 – 2.04 (m, 7H, α-CH and CH<sub>2</sub>), 1.95 (s, 3H, CH<sub>3</sub>), 4.36 – 4.65 (br. m, 1H, NCH) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O): δ = 19.84 (s, CH<sub>3</sub>), 25.60 (d, CH<sub>2</sub>), 27.80 (d, *J*<sub>C,P</sub> = 11.2 Hz, CH<sub>2</sub>), 30.47 (s, CH<sub>2</sub>), 35.94 (d, <sup>1</sup>*J*<sub>C,P</sub> = 141.1 Hz, α-CH), 56.98 (d, *J*<sub>C,P</sub> = 15.3 Hz, NCH), 173.60 (s, C=O) ppm.

**<sup>31</sup>P NMR** (121 MHz, D<sub>2</sub>O): δ = 27.64 and 27.91 ppm. (minor and major isomer)

**Exact mass** (ESI-MS): calculated for C<sub>7</sub>H<sub>13</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 222.0530, found: 222.0503.

**[(1*R*,3*S*)-3-(*N*-Hydroxyacetamido)cyclopentyl]phosphonic acid and [(1*S*,3*R*)-3-(*N*-hydroxyacetamido)cyclopentyl]phosphonic acid (*cis*-3.4).**



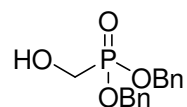
**<sup>1</sup>H NMR** (300 MHz, D<sub>2</sub>O): δ = 1.47 – 2.15 (m, 7H, α-CH and CH<sub>2</sub>), 1.99 (s, 3H, CH<sub>3</sub>), 4.45 – 4.74 (br. m, 1H, NCH) ppm.

**<sup>13</sup>C NMR** (75 MHz, D<sub>2</sub>O): δ = 19.84 (s, CH<sub>3</sub>), 26.89 (d, CH<sub>2</sub>), 29.34 (d, *J*<sub>C,P</sub> = 11.5 Hz, CH<sub>2</sub>), 30.21 (s, CH<sub>2</sub>), 36.66 (d, <sup>1</sup>*J*<sub>C,P</sub> = 140.8 Hz, α-CH), 56.73 (d, *J*<sub>C,P</sub> = 11.8 Hz, NCH), 173.56 (s, C=O) ppm.

**<sup>31</sup>P NMR** (121 MHz, D<sub>2</sub>O): δ = 27.90 and 28.11 ppm. (minor and major isomer)

**Exact mass** (ESI-MS): calculated for C<sub>7</sub>H<sub>13</sub>NO<sub>5</sub>P [M-H]<sup>-</sup>: 222.0530, found: 222.0508.

**Dibenzyl hydroxymethylphosphonate (4.21)**





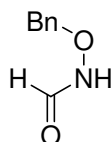
Dibenzyl phosphite (20 mL, 76.27 mmol), triethylamine (10.60 mL, 76.27 mmol), and paraformaldehyde (2.45 g, 130 mmol) were mixed in a reaction vessel. The vessel was tightly sealed and the mixture was mechanically stirred for 12 h at 90 °C. All volatile components were subsequently removed under reduced pressure, and the remaining semisolid was dissolved in 25 mL of CH<sub>2</sub>Cl<sub>2</sub>. The solution was successively washed with saturated aqueous solutions of NH<sub>4</sub>Cl, K<sub>2</sub>CO<sub>3</sub> and again NH<sub>4</sub>Cl, dried with MgSO<sub>4</sub> and the solvent removed under reduced pressure. The residue, a pale-yellow oil, was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/ethyl acetate, 2:1 to CH<sub>2</sub>Cl<sub>2</sub>/acetone 1:1, after al benzyl alcohol had eluted from the column) to yield compound **4.21** (12.48 g, 42.7 mmol, 56%).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 3.92 (d, *J* = 5.6 Hz, 2 H, CH<sub>2</sub>P), 5.06 (m, 4 H, CH<sub>2</sub>Ph), 7.34 (s, 10 H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 57.66 (d, *J* = 160.7 Hz, HOCH<sub>2</sub>), 68.27 (d, *J* = 6.6 Hz OCH<sub>2</sub>Ar), 128.26 (=CH), 128.78 (=CH), 128.86 (=CH), 136.29 (=C) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>15</sub>H<sub>16</sub>O<sub>4</sub>P [M-H]<sup>-</sup>: 291.0785, found: 291.0772.

#### ***N*-(benzyloxy)formamide (4.14)**



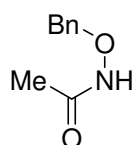
Formic acid (1.18 mL, 31.3 mmol) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (60 mL). To this solution CDI (5.08 g, 31.3 mmol) was added. After stirring for 30 min a suspension of benzylhydroxylamine hydrochloride (5 g, 31.3 mmol) in 31 mL of CH<sub>2</sub>Cl<sub>2</sub> containing Et<sub>3</sub>N (4.35 mL, 31.3 mmol) was added. When TLC revealed disappearance of all the starting material, the reaction mixture was diluted with 150 mL CH<sub>2</sub>Cl<sub>2</sub> and washed six times with 1N HCl (100 mL). The organic layer was dried over MgSO<sub>4</sub>, concentrated under reduced pressure and the residue purified by chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) to yield 4.13 g of *N*-(benzyloxy)formamide **4.14** (yield 87%) as a pale yellow oil.

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): mixture of amide rotamers δ = 4.74 and 4.80 (2 × s, 2 H, CH<sub>2</sub>Ph, minor and major), 7.30 (m, 5 H, arom. H), 7.67 and 8.01 (2 × s, 1 H, C(O)H, major and minor), 9.60 and 10.32 (2 × s, 1 H, NH, minor and major) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): mixture of amide rotamers  $\delta$  = 78.56 and 81.05 (2 × s, OCH<sub>2</sub>Ar, major and minor), 128.79 (=CH), 129.07 (=CH), 129.41 (=CH), 135.09 (=C), 158.35 and 165.08 (2 × s, C(O)H, major and minor) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>8</sub>H<sub>10</sub>NO<sub>2</sub> [M+H]<sup>+</sup>: 152.0712, found: 152.0426.

#### ***N*-(benzyloxy)acetamide (4.15)**



*O*-benzylhydroxylamine hydrochloride (1g, 6.2 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and triethylamine (1 mL, 6.8 mmol) and stirred for 1 h. This suspension was treated with acetyl chloride (0.5 mL, 6.8 mmol) dropwise and stirred for 1.5 h at room temperature. The reaction mixture was diluted with 60 mL of CH<sub>2</sub>Cl<sub>2</sub> and washed with 100 mL of water. The organic solvent was removed under reduced pressure and the residue was purified (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) to yield 0.43 g of *N*-(benzyloxy)acetamide **4.15** (yield 86%) as white crystals.

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): mixture of amide rotamers  $\delta$  = 1.80 and 1.96 (2 × s, 3 H, C(O)CH<sub>3</sub>, major and minor), 4.77 and 4.84 (2 × s, 2 H, CH<sub>2</sub>Ph, minor and major), 7.30 (m, 5 H, arom. H), 8.57 and 9.59 (2 × s, 1 H, NH, minor and major) ppm.

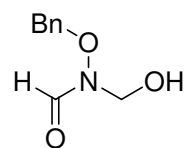
**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): mixture of amide rotamers  $\delta$  = 19.95 and 19.96 (2 × s, CH<sub>3</sub>, minor and major), 78.24 and 79.53 (2 × s, OCH<sub>2</sub>Ar, major and minor), 128.74 (=CH), 128.82 (=CH), 129.32 (=CH), 135.67 (=C), 168.45 and 175.23 (2 × s, C(O)CH<sub>3</sub>, major and minor) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>9</sub>H<sub>12</sub>NO<sub>2</sub> [M+H]<sup>+</sup>: 166.0868, found: 166.0875.

General method for hydroxymethylation of **4.14** and **4.15**.

A mixture of the *N*-benzyloxyamide (5.71 mmol), paraformaldehyde (0.26 g, 8.56 mmol) and *t*BuOK (0.064 g, 0.57 mmol) was heated, in the absence of solvent, at 60 °C during 4 h. The reaction mixture was cooled and diluted with water (80 mL). After extraction with CH<sub>2</sub>Cl<sub>2</sub> (3 × 80 mL) the organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo*. The residue was purified by chromatography (hexane/ethyl acetate, 1:1 for **4.17** or pentane/acetone/Et<sub>3</sub>N, 6:4:0.1 for **4.16**) to give **4.16** and **4.17** in 79% and 48% yield as white solids.

***N*-(benzyloxy)-*N*-(hydroxymethyl)formamide (4.16)**

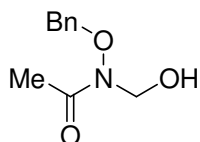


**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 3.91 (br. s, 1 H, OH), 4.76 (s, 2 H, CH<sub>2</sub>Ph), 4.98 (br. s, 2 H, HOCH<sub>2</sub>), 7.37 (m, 5 H, arom. H), 8.08 (s, 1H, C(O)H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 69.55 (s, HOCH<sub>2</sub>, major), 73.10 (s, HOCH<sub>2</sub>, minor), 77.71 (s, CH<sub>2</sub>Ph, minor), 79.81 (s, CH<sub>2</sub>Ph, major), 128.96 (=CH), 129.37 (=CH), 129.87 (=CH), 134.54 (=C), 159.48 (s, C(O)H, minor), 164.27 (s, C(O)H, major) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>9</sub>H<sub>12</sub>NO<sub>3</sub> [M+H]<sup>+</sup>: 182.0817, found: 182.0832.

***N*-(benzyloxy)-*N*-(hydroxymethyl)acetamide (4.17)**



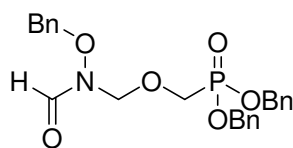
**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 2.06 (s, 3 H, C(O)CH<sub>3</sub>), 3.96 (br. s, 1 H, OH), 4.93 (s, 2 H, CH<sub>2</sub>Ph), 5.03 (br. s, 2 H, HOCH<sub>2</sub>), 7.38 (m, 5 H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 20.86 (s, CH<sub>3</sub>), 71.17 (s, HOCH<sub>2</sub>), 78.45 (s, OCH<sub>2</sub>Ar), 128.94 (=CH), 128.94 (=CH), 128.94 (=CH), 134.65 (=C), 174.26 (s, C(O)CH<sub>3</sub>) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>10</sub>H<sub>14</sub>NO<sub>3</sub> [M+H]<sup>+</sup>: 196.0974, found: 196.0981.

General method for synthesis of ***N*-(benzyloxy)-*N*-(chloromethyl)formamide (4.18)** and ***N*-(benzyloxy)-*N*-(chloromethyl)acetamide (4.19)**

To a cooled solution (10 °C) of **4.16** or **4.17** (2.7 mmol) in 5.5 mL of CH<sub>2</sub>Cl<sub>2</sub> was added thionyl chloride (0.55 mL, 7.5 mmol). After stirring for 30 min, the mixture was evaporated under *vacuum* to yield an oily residue that could be solidified by adding a drop of hexane and connecting the flask to high vacuum. In our hands it was not possible to recrystallize **4.19** from ethyl acetate due to hydrolysis to the alcohol **4.17**. Therefore, **4.19** was directly used in the next step without further purification. The same holds for **4.18**.

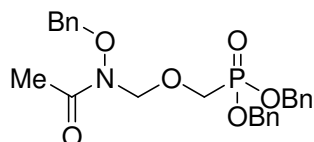
**Dibenzyl(*N*-(*N*-benzyloxyformamido)methoxy)methylphosphonic acid (4.22)**

To a solution of crude **4.18** ( $\pm 0.37$  mmol) in 5 mL of  $\text{CH}_2\text{Cl}_2$  was added a solution **4.21** (0.16 g, 0.54 mmol) in 5 mL of  $\text{CH}_2\text{Cl}_2$ . After stirring for 16 h at room temperature the mixture was evaporated to dryness and purified by column chromatography ( $\text{CH}_2\text{Cl}_2$ /ethyl acetate, 2:1), affording **4.22** (35 mg, 21%) as a clear oil.

**$^1\text{H-NMR}$**  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 3.75 – 3.87 (m, 2H,  $\text{CH}_2\text{P}$ ), 4.74 – 5.15 (m, 8H,  $\text{NCH}_2\text{O}$  and  $\text{PhCH}_2\text{O}$ ), 7.28 – 7.35 (m, 15H, arom. H), 8.07 and 8.15 (br. s and br. s, 1H,  $\text{C(O)H}$ ) ppm.

**$^{13}\text{C-NMR}$**  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 63.60 (d,  $^1J_{\text{C,P}}$  = 171.0 Hz,  $\text{CH}_2\text{P}$ ), 68.24 and 68.32 (2  $\times$  s,  $\text{PhCH}_2$ ), 80.04 ( $\text{NCH}_2\text{O}$ ), 128.33 (=CH), 128.86 (=CH), 129.37 (=CH), 129.80 (=CH), 134.44 (=C), 136.18 (=C), 164.25 ( $\text{C=O}$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{24}\text{H}_{27}\text{NO}_6\text{P}$   $[\text{M}+\text{H}]^+$ : 456.1576, found: 456.1580.

**Dibenzyl(*N*-(*N*-benzyloxyacetamido)methoxy)methylphosphonic acid (4.23)**

To a solution of crude **4.19** ( $\pm 2.5$  mmol) in 2 mL of  $\text{CH}_2\text{Cl}_2$  was added a solution **4.21** (1.46 g, 5 mmol) in 3 mL of  $\text{CH}_2\text{Cl}_2$ . After stirring for 16 h at room temperature the mixture was evaporated to dryness and purified by column chromatography ( $\text{CH}_2\text{Cl}_2$ /ethyl acetate, 2:1) to give **4.23** (0.44 g, 38%) as a clear oil.

**$^1\text{H-NMR}$**  (300 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  = 2.06 (s, 3H,  $\text{C(O)CH}_3$ ), 3.97 (d,  $J$  = 8.8 Hz, 2H,  $\text{CH}_2\text{P}$ ), 4.92 (s, 2H,  $\text{NCH}_2\text{O}$ ), 5.02 (s, 2H,  $\text{PhCH}_2$ ), 5.05 (d,  $J$  = 1.5 Hz, 2H,  $\text{PhCH}_2$ ), 5.08 (d,  $J$  = 2.0 Hz, 2H,  $\text{PhCH}_2$ ), 7.30 – 7.40 (m, 15H, arom. H) ppm.

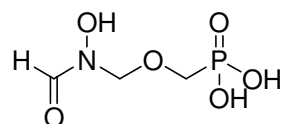
**$^{13}\text{C-NMR}$**  (75 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  = 21.16 ( $\text{CH}_3$ ), 62.76 (d,  $^1J_{\text{C,P}}$  = 219.3 Hz,  $\text{CH}_2\text{P}$ ), 67.70 and 67.78 (2  $\times$  s,  $\text{PhCH}_2$ ), 77.41 ( $\text{NCH}_2\text{O}$ ), 128.48 (=CH), 128.93 (=CH), 129.13 (=CH), 129.15 (=CH), 129.40 (=CH), 130.10 (=CH), 135.35 (=C), 137.03 (d,  $J_{\text{C,P}}$  = 6.1 Hz, =C), 173.18 ( $\text{C=O}$ ) ppm.

**Exact mass** (ESI-MS): calculated for  $C_{25}H_{29}NO_6P$   $[M+H]^+$ : 470.1733, found: 470.1728.

#### General procedure for the benzyl deprotection of 4.22 and 4.23

A solution of compounds **4.22** or **4.23** (140 mg) in MeOH (3 mL) was hydrogenated at atmospheric pressure in the presence of Pd 10 wt. % on activated carbon (70 mg). After 3 h stirring the reaction mixture was filtered over a celite pad. The solvent was removed under *vacuum* and the crude mixture was purified by column chromatography on silica gel ( $CH_2Cl_2$ /MeOH 90:10). Phosphonic acids **4.1** and **4.2** were purified according to the purification of compounds **4.3-4.6**.

**(N-(N-hydroxyformamido)methoxy)methylphosphonic acid (4.1)**: Yield: 34% according to HPLC analysis.



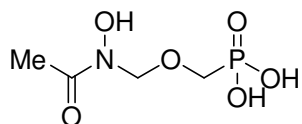
**$^1H$ -NMR** (300 MHz,  $D_2O$ ):  $\delta$  = 3.68 (d,  $J_{H,P}$  = 9.4 Hz, 2H,  $CH_2P$ ), 5.01 (s, 2H,  $OCH_2$ ), 7.94 (s, 1H, C(O)H, minor), 8.21 (s, 1H, C(O)H, major) ppm.

**$^{13}C$ -NMR** (75 MHz,  $D_2O$ ):  $\delta$  = 58.11 (d,  $^1J_{C,P}$  = 153.88 Hz,  $CH_2P$ , major), 63.98 (d,  $^1J_{C,P}$  = 153.88 Hz,  $CH_2P$ , minor), 81.26 (s,  $OCH_2$ , major), 81.40 (s,  $OCH_2$ , minor), 160.19 (s, C=O, minor), 161.032 (s, C=O, major) ppm.

**$^{31}P$ -NMR** (121 MHz,  $D_2O$ ):  $\delta$  = 15.05 ppm.

**Exact mass** (ESI-MS): calculated for  $C_3H_7NO_6P$   $[M-H]^-$ : 184.0010, found: 184.0025.

**(N-(N-hydroxyacetamido)methoxy)methylphosphonic acid (4.2)**: Yield: 43% according to HPLC analysis.



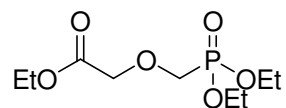
**$^1H$ -NMR** (300 MHz,  $D_2O$ ):  $\delta$  = 2.07 (s, 3H,  $CH_3$ , major), 2.08 (s, 3H,  $CH_3$ , minor), 3.43 (d,  $J_{H,P}$  = 8.9 Hz, 2H,  $CH_2P$ , major), 3.49 (d,  $J_{H,P}$  = 8.9 Hz, 2H,  $CH_2P$ , minor), 4.87 (s, 2H,  $OCH_2$ , major), 4.95 (s, 2H,  $OCH_2$ , minor) ppm.

**$^{13}C$ -NMR** (75 MHz,  $D_2O$ ):  $\delta$  = 19.79 ( $CH_3$ ), 66.38 (d,  $^1J_{C,P}$  = 137.0 Hz,  $CH_2P$ , major), 70.87 (d,  $^1J_{C,P}$  = 137.0 Hz,  $CH_2P$ , minor), 77.78 (s,  $OCH_2$ , major), 77.93 (s,  $OCH_2$ , minor), 175.74 (C=O) ppm.

**<sup>31</sup>P-NMR** (121 MHz, D<sub>2</sub>O):  $\delta$  = 15.16 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>4</sub>H<sub>11</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 200.0324, found: 200.0632.

### Diethyl ((ethoxycarbonyl)methoxy)methylphosphonate (**4.30**)



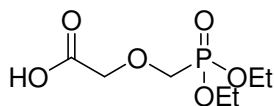
Sodium hydride (400 mg, 11.9 mmol) was added to a solution of diethylhydroxymethyl phosphonate (1 g, 5.9 mmol) in THF (26 mL) and stirred for 1 h. Subsequently, ethyl bromoacetate (1.29 g, 7.7 mmol) was added and, after 3 h, the reaction was quenched with sat. NH<sub>4</sub>Cl (aq.). EtOAc was added, the two phases separated and the aqueous layer further extracted with three portions of EtOAc (100 mL). The combined organic layers were dried over MgSO<sub>4</sub> and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95:5) to give **4.30** (1.21 g, 80%) as an oil.

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.25 (t,  $J$  = 7.0 Hz, 6H, C(O)OCH<sub>2</sub>CH<sub>3</sub>), 1.32 (t,  $J$  = 7.0 Hz, 6H, POCH<sub>2</sub>CH<sub>3</sub>), 3.91 (d,  $J_{H,P}$  = 8.5 Hz, 2H, CH<sub>2</sub>P), 4.12 – 4.22 (m, 8H, C(O)CH<sub>2</sub>O and POCH<sub>2</sub>CH<sub>3</sub>) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 14.37 (C(O)OCH<sub>2</sub>CH<sub>3</sub>), 16.64 (d,  $^3J_{C,P}$  = 5.8 Hz, POCH<sub>2</sub>CH<sub>3</sub>), 61.19 (C(O)OCH<sub>2</sub>CH<sub>3</sub>), 62.79 (d,  $^2J_{C,P}$  = 6.3 Hz, POCH<sub>2</sub>CH<sub>3</sub>), 65.30 (d,  $^1J_{C,P}$  = 165.5 Hz, CH<sub>2</sub>P), 69.66 (d,  $^3J_{C,P}$  = 11.2 Hz, C(O)CH<sub>2</sub>O), 169.79 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>9</sub>H<sub>20</sub>O<sub>6</sub>P [M+H]<sup>+</sup>: 255.0998, found: 255.0987.

### 2-((Ethoxyphosphono)methoxy)acetic acid (**4.32**)



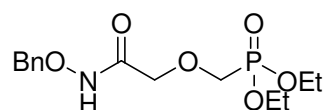
A solution of **4.30** (1.21 g, 4.8 mmol) in 4.8 mL 1N aq. NaOH was stirred for 2 h at 30 °C. When the hydrolysis was finished, as indicated by TLC, the reaction mixture was neutralized with 1N HCl until PH  $\approx$  9. The desired carboxylic acid was extracted with CH<sub>2</sub>Cl<sub>2</sub> (5  $\times$  50 mL) and EtOAc (3  $\times$  50 mL). The combined organic layers were dried over MgSO<sub>4</sub> and concentrated *in vacuo*. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ HCOOH, 95:5:0.1) afforded **4.31** (1.0 g, 94%). The carboxylic acid was visualized on TLC with bromocresol green.

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.36 (t, *J* = 7.0 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 3.98 (d, *J*<sub>H,P</sub> = 8.2 Hz, 2H, CH<sub>2</sub>P), 4.16 – 4.26 (m, 6H, C(O)CH<sub>2</sub>O and OCH<sub>2</sub>CH<sub>3</sub>), 6.79 (br. s, 1H, OH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.61 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.34 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.3 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 65.40 (d, <sup>1</sup>*J*<sub>C,P</sub> = 165.8 Hz, CH<sub>2</sub>P), 69.74 (d, <sup>3</sup>*J*<sub>C,P</sub> = 10.1 Hz, C(O)CH<sub>2</sub>O), 171.91 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>7</sub>H<sub>16</sub>O<sub>6</sub>P [M+H]<sup>+</sup>: 227.0685, found: 227.0683.

#### Diethyl ((benzyloxycarbamoyl)methoxy)methylphosphonate (**4.32**)



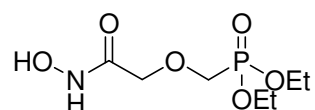
Compound **4.31** (0.80 g, 3.5 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4 mL). To this solution 1.1 eq. CDI (0.60 g, 3.7 mmol) was added. After stirring for 0.5 h, a suspension of benzylhydroxylamine hydrochloride (0.59 g, 3.7 mmol) in 4 mL of CH<sub>2</sub>Cl<sub>2</sub> containing Et<sub>3</sub>N (0.38 g, 3.7 mmol) was added. After disappearance of all starting material as indicated by TLC, the reaction mixture was diluted with 50 mL CH<sub>2</sub>Cl<sub>2</sub> and washed with 1N HCl (60 mL). The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95:5) afforded **4.32** as an oil in 83% yield (970 mg).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.31 (t, *J* = 7.0 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 3.76 (d, *J*<sub>H,P</sub> = 8.8 Hz, 2H, CH<sub>2</sub>P), 4.07 – 4.17 (m, 6H, C(O)CH<sub>2</sub>O and OCH<sub>2</sub>CH<sub>3</sub>), 4.96 (s, 2H, PhCH<sub>2</sub>O), 7.34 – 7.44 (m, 5H, arom. H), 9.46 (br. s, 1H, NH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.65 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.01 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.6 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 66.19 (d, <sup>1</sup>*J*<sub>C,P</sub> = 168.4 Hz, CH<sub>2</sub>P), 72.33 (d, <sup>3</sup>*J*<sub>C,P</sub> = 10.1 Hz, CH<sub>2</sub>), 78.59 (PhCH<sub>2</sub>O), 128.78 (=CH), 128.95 (=CH), 129.39 (=CH), 135.0 (=C), 168.91 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>14</sub>H<sub>23</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 332.1263, found: 332.1272.

#### Diethyl ((hydroxycarbamoyl)methoxy)methylphosphonate (**4.33**)



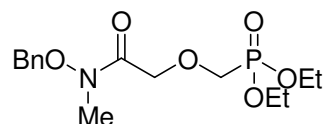
Benzyl deprotection of **4.32** was essentially performed as described for **4.1** and **4.2**, yielding the title compound in 80% yield (92 mg).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.30 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 3.85 (m, 2H, CH<sub>2</sub>P), 4.14 (m, 6H, C(O)CH<sub>2</sub>O and OCH<sub>2</sub>CH<sub>3</sub>), 10.02 (br. s, 1H, NH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.59 (d, <sup>3</sup>J<sub>C,P</sub> = 5.5 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 63.23 (d, <sup>2</sup>J<sub>C,P</sub> = 6.3 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 65.91 (d, <sup>1</sup>J<sub>C,P</sub> = 166.7 Hz, CH<sub>2</sub>P), 71.57 (d, <sup>3</sup>J<sub>C,P</sub> = 10.0 Hz, C(O)CH<sub>2</sub>O), 166.24 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>8</sub>H<sub>17</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 254.0794, found: 254.0806.

#### Diethyl ((*N*-(benzyloxy)-*N*-methylcarbamoyl)methoxy)methylphosphonate (**4.34**)



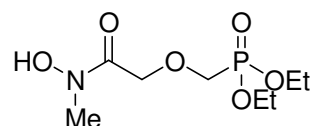
To solution of **4.32** (0.51 g, 1.5 mmol) in 16 mL of THF was added sodium hydride (67 mg, 1.7 mmol) and methyl iodide (0.24 g, 1.7 mmol). The mixture was stirred overnight at room temperature, quenched with a saturated solution of NH<sub>4</sub>Cl (40 mL) and extracted with ether (3 × 40 mL). The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95:5) afforded **4.34** (300 mg, 56%).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.30 (t, *J* = 7.0 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 3.17 (s, 3H, NCH<sub>3</sub>), 3.83 (d, *J*<sub>H,P</sub> = 8.2 Hz, 2H, CH<sub>2</sub>P), 4.09 – 4.19 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.25 (s, 2H, C(O)CH<sub>2</sub>O), 4.77 (s, 2H, PhCH<sub>2</sub>O), 7.26 – 7.37 (m, 5H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.63 (d, <sup>3</sup>J<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 33.73 (NCH<sub>3</sub>), 62.71 (d, <sup>2</sup>J<sub>C,P</sub> = 6.6 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 65.11 (d, <sup>1</sup>J<sub>C,P</sub> = 164.7 Hz, CH<sub>2</sub>P), 69.91 (d, <sup>3</sup>J<sub>C,P</sub> = 11.5 Hz, C(O)CH<sub>2</sub>O), 76.53 (PhCH<sub>2</sub>O), 129.00 (=CH), 129.42 (=CH), 129.65 (=CH), 134.28 (=C), 171.13 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>15</sub>H<sub>25</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 346.1420, found: 346.1408.

#### Diethyl ((*N*-hydroxy-*N*-methylcarbamoyl)methoxy)methylphosphonate (**4.35**)





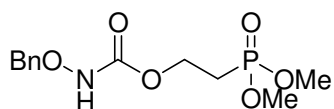
Benzyl deprotection of **4.34** was essentially performed as described for **4.1** and **4.2**, yielding the title compound in 89% yield (140 mg).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.31 (t, *J* = 7.0 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 3.19 (s, 3H, NCH<sub>3</sub>), 3.91 (d, *J*<sub>H,P</sub> = 8.2 Hz, 2H, CH<sub>2</sub>P), 4.09 – 4.19 (m, 4H, OCH<sub>2</sub>CH<sub>3</sub>), 4.41 (s, 2H, C(O)CH<sub>2</sub>O), 9.38 (br. s, 1H, OH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 16.57 (d, <sup>3</sup>*J*<sub>C,P</sub> = 5.8 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 36.03 (NCH<sub>3</sub>), 63.22 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.6 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 65.02 (d, <sup>1</sup>*J*<sub>C,P</sub> = 166.1 Hz, CH<sub>2</sub>P), 70.01 (d, <sup>3</sup>*J*<sub>C,P</sub> = 11.8 Hz, C(O)CH<sub>2</sub>O), 169.83 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>8</sub>H<sub>19</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 256.0950, found: 256.1052.

#### Dimethyl 2(*N*-benzyloxycarbonyloxy)ethylphosphonate (**4.37**)



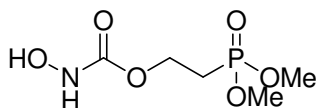
The title compound was prepared in 73% yield from dimethyl 2-hydroxyethylphosphonate (**4.36**) using a similar procedure as described for **4.32**.

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 2.13 (dt, *J*<sub>H,H</sub> = 7.3 Hz and *J*<sub>H,P</sub> = 18.8 Hz, 2H, CH<sub>2</sub>P), 3.68 (d, *J* = 11.1 Hz, 6H, OCH<sub>3</sub>), 4.30 (dt, *J*<sub>H,H</sub> = 7.6 Hz and *J*<sub>H,P</sub> = 12.9 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 4.81 (s, 2H, PhCH<sub>2</sub>O), 7.29 – 7.34 (m, 5H, arom. H), 8.15 (br. s, 1H, NH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 25.42 (d, <sup>1</sup>*J*<sub>C,P</sub> = 140.5 Hz, CH<sub>2</sub>P), 52.73 (d, <sup>2</sup>*J*<sub>C,P</sub> = 6.6 Hz, OCH<sub>3</sub>), 59.79 (CH<sub>2</sub>CH<sub>2</sub>P), 78.78 (PhCH<sub>2</sub>O), 128.69 (=CH), 128.78 (=CH), 129.32 (=CH), 135.68 (=C), 157.16 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>12</sub>H<sub>19</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 304.0950, found: 304.0947.

#### Dimethyl 2(*N*-hydroxycarbonyloxy)ethylphosphonate (**4.38**)



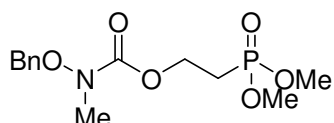
Benzyl deprotection of **4.37** was essentially performed as described for **4.1** and **4.2**, yielding the title compound in 98% yield (350 mg).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 2.21 (dt,  $J_{\text{H,H}} = 7.0$  Hz and  $J_{\text{H,P}} = 18.8$  Hz, 2H, CH<sub>2</sub>P), 3.77 (d,  $J = 11.1$  Hz, 6H, OCH<sub>3</sub>), 4.40 (dt,  $J_{\text{H,H}} = 7.0$  Hz and  $J_{\text{H,P}} = 15.3$  Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 7.40 (br. s, 1H, NH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 25.45 (d,  $^1J_{\text{C,P}} = 141.4$  Hz, CH<sub>2</sub>P), 52.96 (d,  $^2J_{\text{C,P}} = 6.6$  Hz, OCH<sub>3</sub>), 59.82 (CH<sub>2</sub>CH<sub>2</sub>P), 158.38 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>5</sub>H<sub>13</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 214.0481, found: 214.0479.

### Dimethyl 2(*N*-benzyloxy-*N*-methylcarbonyloxy)ethylphosphonate (4.39)



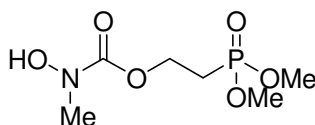
The title compound was prepared in 68% yield from **4.37** using a similar procedure as described for **4.34**.

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 2.03 (dt,  $J_{\text{H,H}} = 7.0$  Hz and  $J_{\text{H,P}} = 18.8$  Hz, 2H, CH<sub>2</sub>P), 2.91 (s, 3H, NCH<sub>3</sub>), 3.56 (d,  $J = 10.8$  Hz, 6H, OCH<sub>3</sub>), 4.20 (dt,  $J_{\text{H,H}} = 7.3$  Hz and  $J_{\text{H,P}} = 13.2$  Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>P), 4.69 (s, 2H, PhCH<sub>2</sub>O), 7.15 – 7.25 (m, 5H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 25.33 (d,  $^1J_{\text{C,P}} = 140.5$  Hz, CH<sub>2</sub>P), 36.89 (NCH<sub>3</sub>), 52.52 (d,  $^2J_{\text{C,P}} = 6.3$  Hz, OCH<sub>3</sub>), 60.08 (CH<sub>2</sub>CH<sub>2</sub>P), 76.65 (PhCH<sub>2</sub>O), 128.52 (=CH), 128.70 (=CH), 129.48 (=CH), 135.47 (=C), 157.06 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>13</sub>H<sub>21</sub>NO<sub>6</sub>P [M+H]<sup>+</sup>: 318.1107, found: 318.1111.

### Dimethyl-2(*N*-hydroxy-*N*-methylcarbonyloxy)ethylphosphonate (4.40)



Benzyl deprotection of **4.39** was essentially performed as described for **4.1** and **4.2**, yielding the title compound in 97% yield (300 mg).

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 2.09 (dt,  $J_{\text{H,H}} = 6.2$  Hz and  $J_{\text{H,P}} = 18.8$  Hz, 2H, CH<sub>2</sub>P), 3.08 (s, 3H, NCH<sub>3</sub>), 3.64 (d,  $J = 10.8$  Hz, 6H, OCH<sub>3</sub>), 4.40 (dt,  $J_{\text{H,H}} = 6.5$  Hz and  $J_{\text{H,P}} = 13.8$  Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>P) ppm.

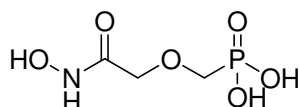
**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 25.27 (d,  $^1J_{\text{C,P}} = 140.5$  Hz, CH<sub>2</sub>P), 38.34 (NCH<sub>3</sub>), 52.82 (d,  $^2J_{\text{C,P}} = 6.3$  Hz, OCH<sub>3</sub>), 59.76 (CH<sub>2</sub>CH<sub>2</sub>P), 157.22 (C=O) ppm.

**Exact mass** (ESI-MS): calculated for  $C_6H_{15}NO_6P$   $[M+H]^+$ : 228.0637, found: 228.0624.

General method for the phosphonate deprotection towards compounds **4.3**, **4.4**, **4.5** and **4.6**.

Esters **4.33**, **4.35**, **4.38** or **4.40** (0.39 mmol) were dissolved in  $CH_2Cl_2$  (1.1 mL) and treated dropwise with 2 eq. of TMSBr (0.77 mmol, 83 mg) under  $N_2$ . The reaction mixture was stirred for 2 h at room temperature. After completion of the reaction the volatile compounds were removed *in vacuo* to give the corresponding phosphonic acids. All final compounds were purified using a preparative HPLC system on a C18 column (5 $\mu$ m, Phenomenex, Luna, 250 x 21.2 mm) with a linear gradient of acetonitrile in 5 mM  $NH_4OAc$  solution over 20 min at a flow rate of 17.5 mL/min. The purity of all target compounds was assessed by analytical HPLC (5 $\mu$ m, Phenomenex, C18(2), 250 x 4.6 mm) using the same gradient at a flow rate of 1 mL/min. All final compounds were obtained as hygroscopic foams or slight yellow oils after lyophilisation. For compounds **4.3** and **4.4**, this procedure was not very efficient since the target compounds showed poor retention (elution time: 3.6 min). Nevertheless it permitted to produce a pure fraction.

**((Hydroxycarbamoyl)methoxy)methylphosphonic acid (4.3):** Yield: 52% according to HPLC analysis.



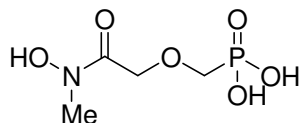
**$^1H$ -NMR** (300 MHz,  $D_2O$ ):  $\delta$  = 3.49 (d,  $J_{H,P}$  = 9.1 Hz, 2H,  $CH_2P$ ), 3.92 (s, 2H,  $C(O)CH_2O$ , minor), 4.07 (s, 2H,  $C(O)CH_2O$ , major) ppm.

**$^{13}C$ -NMR** (75 MHz,  $D_2O$ ):  $\delta$  = 69.47 (d,  $^1J_{C,P}$  = 152.3 Hz,  $CH_2P$ ), 70.76 (d,  $^3J_{C,P}$  = 13.3 Hz,  $C(O)CH_2OO$ ), 169.31 (C=O) ppm.

**$^{31}P$ -NMR** (121 MHz,  $D_2O$ ):  $\delta$  = 15.58 ppm.

**Exact mass** (ESI-MS): calculated for  $C_3H_7NO_6P$   $[M-H]^-$ : 184.0010, found: 184.0456.

**((*N*-hydroxy-*N*-methylcarbamoyl)methoxy)methylphosphonic acid (4.4):** Yield: 45% according to HPLC analysis.



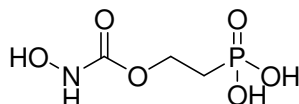
**<sup>1</sup>H-NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 3.16 (s, 3H, NCH<sub>3</sub>, major), 3.22 (s, 3H, NCH<sub>3</sub>, minor), 3.51 (d,  $J_{\text{H,P}}$  = 8.5 Hz, 2H, CH<sub>2</sub>P), 4.25 (s, 2H, C(O)CH<sub>2</sub>O, minor), 4.38 (s, 2H, C(O)CH<sub>2</sub>O, major) ppm.

**<sup>13</sup>C-NMR** (75 MHz, D<sub>2</sub>O):  $\delta$  = 36.09 (NCH<sub>3</sub>), 68.40 (d,  $^1J_{\text{C,P}}$  = 157.5 Hz, CH<sub>2</sub>P), 69.37 (d,  $^3J_{\text{C,P}}$  = 11.5 Hz, C(O)CH<sub>2</sub>O), 171.73 (C=O) ppm.

**<sup>31</sup>P-NMR** (121 MHz, D<sub>2</sub>O):  $\delta$  = 15.42 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>4</sub>H<sub>9</sub>NO<sub>6</sub>P [M-H]<sup>-</sup>: 198.0166, found: 198.0160.

**2(*N*-hydroxycarbonyloxy)ethylphosphonic acid (4.5):** Yield: 71 mg (quantitative).



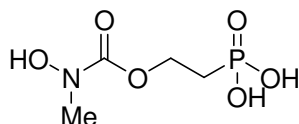
**<sup>1</sup>H-NMR** (300 MHz, MeOD):  $\delta$  = 2.14 (dt,  $J_{\text{H,H}}$  = 7.0 Hz and  $J_{\text{H,P}}$  = 19.1 Hz, 2H, CH<sub>2</sub>P), 4.31 (dt,  $J_{\text{H,H}}$  = 7.6 Hz and  $J_{\text{H,P}}$  = 10.6 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>P) ppm.

**<sup>13</sup>C-NMR** (75 MHz, MeOD):  $\delta$  = 27.51 (d,  $^1J_{\text{C,P}}$  = 137.0 Hz, CH<sub>2</sub>P), 59.97 (CH<sub>2</sub>CH<sub>2</sub>P), 159.15 (C=O) ppm.

**<sup>31</sup>P-NMR** (121 MHz, MeOD):  $\delta$  = 25.63 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>3</sub>H<sub>8</sub>NO<sub>6</sub>P [M-H]<sup>-</sup>: 184.0010, found: 184.0007.

**2(*N*-hydroxy-*N*-methylcarbonyloxy)ethylphosphonic acid (4.6):** Yield: 153 mg (quantitative).

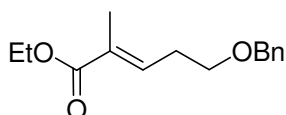


**<sup>1</sup>H-NMR** (300 MHz, MeOD):  $\delta$  = 2.16 (m, 2H, CH<sub>2</sub>P), 3.16 (s, 3H, NCH<sub>3</sub>), 4.32 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>P) ppm.

**<sup>13</sup>C-NMR** (75 MHz, MeOD):  $\delta$  = 27.43 (d,  $^1J_{\text{C,P}}$  = 138.2 Hz, CH<sub>2</sub>P), 37.68 (NCH<sub>3</sub>), 60.68 (CH<sub>2</sub>CH<sub>2</sub>P), 157.74 (C=O) ppm.

**<sup>31</sup>P-NMR** (121 MHz, MeOD):  $\delta$  = 25.92 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>4</sub>H<sub>9</sub>NO<sub>6</sub>P [M-H]<sup>-</sup>: 198.0166, found: 198.0173.

**(E)-Ethyl 5-(benzyloxy)-2-methylpent-2-enoate (5.18)**

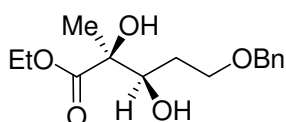
To a cold solution (0 °C) of 3-(benzyloxy)propan-1-ol (1.59 mL, 10 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added Dess-Martin periodinane 15 wt% (50 mL, 25 mmol). One drop of H<sub>2</sub>O was added and the reaction mixture was stirred at room temperature for 1 h. After this time the reaction mixture was diluted with saturated aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (100mL). The mixture was extracted three times with CH<sub>2</sub>Cl<sub>2</sub> (100mL). The combined organic layers were dried over MgSO<sub>4</sub> and concentrated *in vacuo* to give crude 3-(benzyloxy)propanal **5.17** (R<sub>f</sub> = 0.33 in EtOAc/hexane 1:4), which was used without further purification.

The residue thus obtained was dissolved in 50 mL of toluene. Ethylidenetriphenylphosphorane (5 g, 11 mmol) was added and the reaction mixture was heated under reflux for 30 min, after which time the solvent was removed by evaporation. The residue was triturated with pentane and the resulting solid was removed by filtration. The filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:99 to 2:98) to provide 1.69 g of **5.18** (R<sub>f</sub> = 0.54 in EtOAc/hexane 2:8) as an oil which predominantly consisted of the *E*-isomer based on <sup>1</sup>H NMR analysis. The yield of both steps was 68.3%.

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>): δ = 1.29 (dt, *J* = 0.6 Hz and 7.0 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.85 (d, *J* = 0.6 Hz, 3H, CH<sub>3</sub>C=C), 2.50 (virtual q, AB system, *J* = 6.9 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>CH), 3.57 (t, *J* = 6.7 Hz, 2H, OCH<sub>2</sub>CH<sub>2</sub>), 4.19 (dq, *J* = 0.6 Hz and 7.0 Hz, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.53 (s, 2H, OCH<sub>2</sub>Ph), 6.78 (dt, *J* = 0.6 Hz and 7.3 Hz, 1H, CH<sub>2</sub>CH=C), 7.25 – 7.76 (m, 5H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>): δ = 17.28, 19.02, 34.16, 65.20, 73.36, 77.74, 132.37, 133.14, 134.16, 135.06, 137.79, 142.96, 168.00 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>15</sub>H<sub>20</sub>NaO<sub>3</sub> [M+Na]<sup>+</sup>: 271.1309, found: 271.1290.

**(2S,3R)-Ethyl 5-(benzyloxy)-2,3-dihydroxy-2-methylpentanoate (5.19)**

MeSO<sub>2</sub>NH<sub>2</sub> was added to a solution of AD-mix-β (3.61 g) in *t*-butyl alcohol (13 mL) and H<sub>2</sub>O (13 mL). Stirring was continued at room temperature until both phases were clear. Then the reaction mixture was cooled to 0 °C, whereupon the inorganic salts partially precipitated. The olefin (*E*)-ethyl 5-(benzyloxy)-2-methylpent-2-enoate **5.18** (0.64 g, 2.58 mmol) was added at once and the reaction mixture was stirred vigorously at 0 °C until TLC revealed the absence of the starting olefin (24 h). The reaction was quenched at 0 °C by addition of sodium sulfite and then warmed to room temperature and stirred for 45 min. The reaction mixture was extracted five times with ethyl acetate (5 × 100 mL). The combined organic layers were washed with 2 N KOH (100 mL) to remove most of the sulphonamide. Organic solvents were dried over MgSO<sub>4</sub> and removed *in vacuo*. The residue was purified by flash chromatography (silica gel, EtOAc/hexane 2:98, 2:8, 4:6) (*R*<sub>f</sub> = 0.35 in EtOAc/hexane 4:6) to give the product **5.19** (0.48 g, 1.71 mmol) as a clear oil in 66% yield and with an enantiomeric excess of 92%.

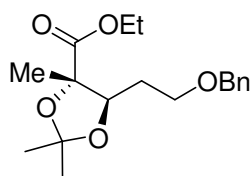
[α]<sub>25</sub><sup>D</sup> = + 6.4 (in EtOH)

<sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ = 1.17 (2 × 3 H, m, OCH<sub>2</sub>CH<sub>3</sub> and CH<sub>3</sub>C), 1.48 (1 H, m, OCH<sub>2</sub>CH<sub>2</sub>), 1.75 (1 H, m, OCH<sub>2</sub>CH<sub>2</sub>), 3.52 (2 H, m, OCH<sub>2</sub>CH<sub>2</sub>), 3.65 (1 H, virtual septet, *J* = 2.1 Hz, 8.2 Hz and 9.2 Hz, HCOH), 4.06 (2 H, m, OCH<sub>2</sub>CH<sub>3</sub>), 4.43 (2 H, s, OCH<sub>2</sub>Ph), 4.57 (1 H, d, *J* = 8.2 Hz, HCOH), 4.81 (1 H, s, MeCOH), 7.23 – 7.36 (5 H, m, arom. H) ppm.

<sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ = 14.15, 21.91, 30.46, 60.25, 66.97, 71.87, 72.09, 77.36, 127.35, 127.44, 128.26, 138.77, 175.13 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>15</sub>H<sub>22</sub>NaO<sub>5</sub> [M+Na]<sup>+</sup>: 305.1365, found: 305.1351.

**(4*S*,5*R*)-Ethyl 5-(2-(benzyloxy)ethyl)-2,2,4-trimethyl-1,3-dioxolane-4-carboxylate (5.20)**



A solution of (2*S*,3*R*)-ethyl 5-(benzyloxy)-2,3-dihydroxy-2-methylpentanoate **5.19** (0.48 g, 1.7 mmol) in anhydrous acetone (20 mL) was treated with 2,2-dimethoxypropane (0.88 g, 8.5 mmol, 1.08 mL) and TsOH (0.05 eq), and the

resulting mixture was stirred at room temperature for 4 h. Then the reaction mixture was poured into 80 mL of a 7% NaHCO<sub>3</sub> solution and extracted thrice with 80 mL of CH<sub>2</sub>Cl<sub>2</sub>. Evaporation of the organic solution afforded a crude product consisting of practically pure **5.20** which was used in the next step after flash chromatography (silica gel, EtOAc/hexane 3:97, R<sub>f</sub> = 0.10). The yield was 94%.

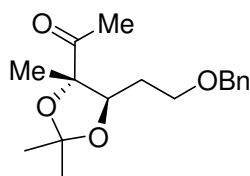
$[\alpha]_D^{25} = +36.0$  (in CHCl<sub>3</sub>)

**<sup>1</sup>H-NMR** (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.27 (t,  $J$  = 7.3 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.34 (s, 3H, CH<sub>3</sub>C), 1.39 (s, 3H, CH<sub>3</sub>C), 1.46 (s, 3H, CH<sub>3</sub>C), 1.93 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>), 3.63 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>), 4.19 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>),  $\delta$  4.36 (dd,  $J$  = 3.5 Hz and 9.7 Hz, 1H, CH), 4.50 (d, AB system,  $J$  = 11.7 Hz, 1H, OCH<sub>2</sub>Ph), 4.77 (d, AB system,  $J$  = 12.0 Hz, 1H, OCH<sub>2</sub>Ph), 7.27 – 7.34 (m, 5H, arom. H) ppm.

**<sup>13</sup>C-NMR** (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 14.34, 20.13, 25.74, 28.47, 30.33, 61.62, 67.59, 73.16, 82.62, 109.22, 127.79, 128.59, 138.61, 163.24, 173.29 ppm.

**Exact mass** (ESI-MS): calculated for C<sub>18</sub>H<sub>26</sub>NaO<sub>5</sub> [M+Na]<sup>+</sup>: 345.1677, found: 345.1666.

**1-((4*S*,5*R*)-5-(2-(benzyloxy)ethyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl)ethanone**  
**(5.22)**



A solution of ester (4*S*,5*R*)-ethyl 5-(2-(benzyloxy)ethyl)-2,2,4-trimethyl-1,3-dioxolane-4-carboxylate **5.20** (0.51 g, 1.60 mmol) and KOH (0.13g, 2.23 mmol) in MeOH (0.5 mL) and H<sub>2</sub>O (0.2 mL) was stirred at reflux for 1 h. The reaction mixture was cooled and extracted with Et<sub>2</sub>O (80 mL). The aqueous layer was acidified with concentrated HCl and extracted thrice with Et<sub>2</sub>O (3 × 80 mL). The combined extracts were washed with brine, dried over MgSO<sub>4</sub> and concentrated under reduced pressure to yield 0.44 g of crude acid. The yield was 93%.

MeLi (2.04 mL of a 1.6 M solution in ether, 3.25 mmol) was added drop wise to a solution of the above acid (0.44 g, 1.49 mmol) in dry THF (12 mL) at 0 °C. The reaction mixture was allowed to warm up to 25 °C over the period of 1 h and quenched by transferring it into an ice-1 N HCl mixture by cannula. The reaction mixture was extracted thrice with Et<sub>2</sub>O (3 × 80 mL). The combined extracts were

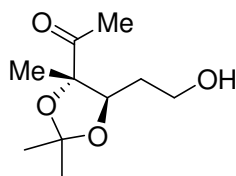
washed with brine, dried over  $\text{MgSO}_4$  and concentrated under reduced pressure to yield a mixture of the methyl keton and the corresponding tertiary alcohol. Flash chromatography gave 0.29 g (yield 69%) of **5.22** ( $R_f = 0.13$  in EtOAc/hexane 3:97).

**$^1\text{H-NMR}$**  (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 1.22$  (s, 3H,  $\text{CH}_3$ ), 1.39 (s, 3H,  $\text{CH}_3$  of isopropylidene), 1.47 (s, 3H,  $\text{CH}_3$  of isopropylidene), 1.86 (m, 2H,  $\text{OCH}_2\text{CH}_2$ ), 2.27 (s, 3H,  $\text{CH}_3\text{C=O}$ ), 3.59 (m, 2H,  $\text{OCH}_2\text{CH}_2$ ), 4.36 (dd,  $J = 3.5$  Hz and 9.4 Hz, 1H, CH), 4.48 (d, AB system,  $J = 12.0$  Hz, 1H,  $\text{OCH}_2\text{Ph}$ ), 4.52 (d, AB system,  $J = 12.0$  Hz, 1H,  $\text{OCH}_2\text{Ph}$ ), 7.34 (m, 5H, arom. H) ppm.

**$^{13}\text{C-NMR}$**  (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.34, 20.13, 25.74, 28.47, 30.32, 61.64, 67.60, 73.17, 82.62, 109.24, 127.80, 128.60, 138.59, 163.23, 173.31$  ppm.

**Exact mass** (ESI-MS): calculated for  $\text{C}_{17}\text{H}_{24}\text{NaO}_4$   $[\text{M}+\text{Na}]^+$ : 315.1572, found: 315.1578.

#### 1-((4*S*,5*R*)-5-(2-Hydroxyethyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl)ethanone (**5.23**)



Compound **5.22** (0.29 g, 0.99 mmol) was dissolved in methanol (15 mL) under nitrogen atmosphere and Pd on carbon (10%) (40 mg) was added to the solution. The suspension was drawn *in vacuo* by the pump. A balloon with  $\text{H}_2$  gas was placed on the recipient and the suspension was stirred for 24 h. After spotting ( $R_f = 0.31$  in EtOAc/hexane 4:6) of the reaction a balloon with fresh  $\text{H}_2$  gas was placed on the recipient. When the reaction was finished the reaction mixture was filtered and concentrated under reduced pressure. Flash chromatography (silica gel, EtOAc/hexane 8:2 > 4:6,  $R_f = 0.29$  in EtOAc/hexane 4:6) gave 0.15 g of product **5.23** (yield 71%).

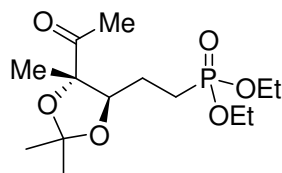
**$^1\text{H-NMR}$**  (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 1.25$  (s, 3H,  $\text{CH}_3$ ), 1.38 (s, 3H,  $\text{CH}_3$  of isopropylidene), 1.48 (s, 3H,  $\text{CH}_3$  of isopropylidene), 1.85 (m, 2H,  $\text{CH}_2\text{CH}_2\text{OH}$ ), 2.29 (s, 3H,  $\text{CH}_3\text{C=O}$ ), 3.79 (m, 2H,  $\text{CH}_2\text{CH}_2\text{OH}$ ), 4.14 (dd,  $J = 4.5$  Hz and 8.6 Hz, 1H, CH) ppm.

**$^{13}\text{C-NMR}$**  (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 19.56, 25.88, 26.18, 28.58, 32.40, 61.17, 77.75, 87.60, 109.13, 212.32$  ppm.



**Exact mass** (ESI-MS): calculated for  $C_{10}H_{18}NaO_4$   $[M+Na]^+$ : 225.1102, found: 225.1106.

**Diethyl 2-((4*S*,5*R*)-5-acetyl-2,2,5-trimethyl-1,3-dioxolan-4-yl)ethylphosphonate (5.25)**



To a solution of compound **5.23** (0.15 g, 0.71 mmol) and imidazole (0.11 g, 1.43 mmol) in 7 mL of dry toluene was added with stirring under nitrogen freshly distilled chlorodiphenylphosphane (0.21 g, 0.93 mmol). The resulting cloudy mixture was treated with iodine in small portions at room temperature, and stirring was continued until complete conversion. An equal volume of 2 N NaOH was added, the mixture was shaken thoroughly for 5 min, and more iodine was added until the brown colour persisted in the organic phase. The layers were separated, and the organic phase was washed with saturated sodium thiosulfate and water, and then dried with  $MgSO_4$ . The colourless material obtained after concentration of the solution *in vacuo* proved of sufficient purity and, because of its presumed lability, the product was used in the next step without further purification.

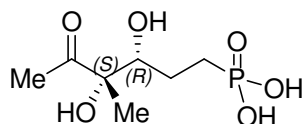
A solution of crude iodide **5.24** in 0.6 mL triethyl phosphite was heated at 130 °C under nitrogen for 12 h. The excess triethylphosphite and other volatile compounds were removed under reduced pressure. The residue was purified by flash chromatography (silica gel, EtOAc/hexane 4:6,  $R_f$  = 0.32 in  $CH_2Cl_2$ /MeOH 9:1) to yield **5.25** as colourless oil (yield 81%).

**$^1H$ -NMR** (300 MHz,  $CDCl_3$ ):  $\delta$  = 1.22 (s, 3H,  $CH_3$ ), 1.32 (t,  $J$  = 7.0 Hz, 6H,  $P(O)OCH_2CH_3$ ), 1.36 (s, 3H,  $CH_3$  of isopropylidene), 1.46 (s, 3H,  $CH_3$  of isopropylidene), 1.90 (m, 4H,  $CH_2CH_2P$ ), 2.28 (s, 3H,  $CH_3C=O$ ), 3.99 (m, 1H, CH), 4.09 (m, 4H,  $OCH_2CH_3$ ) ppm.

**$^{13}C$ -NMR** (75 MHz,  $CDCl_3$ ):  $\delta$  = 16.69, 19.28, 23.14, 23.38, 25.70, 26.18, 28.59, 29.93, 61.83, 78.59, 87.45, 211.84 ppm.

**Exact mass** (ESI-MS): calculated for  $C_{14}H_{27} NaO_6P_1$   $[M+Na]^+$ : 345.1411, found: 345.1444.

### (3*S*,4*R*)-3,4-Dihydroxy-4-methyl-5-oxohexylphosphonic acid (**5.1**)



The ester **5.22** (0.19 g, 0.58 mmol) was dissolved in 2 mL CH<sub>2</sub>Cl<sub>2</sub> and treated drop wise with TMSBr (0.35 g, 2.31 mmol). The reaction mixture was stirred under nitrogen for 2 h by room temperature. After removal of the volatile compounds *in vacuo* the residue was dissolved in water. The residue was purified on RP-HPLC in a same way as described for the other analogues, to yield **5.1** as a slight yellow powder in almost quantitative yield.

**<sup>1</sup>H-NMR** (300 MHz, D<sub>2</sub>O):  $\delta$  = 1.15 (s, 3H, CH<sub>3</sub>), 1.42 (m, 2H, PCH<sub>2</sub>), 1.67 (m, 2H, PCH<sub>2</sub>CH<sub>2</sub>), 2.13 (s, 3H, CH<sub>3</sub>C=O), 3.76 (m, 1H, CH) ppm.

**<sup>13</sup>C-NMR** (75 MHz, D<sub>2</sub>O): 20.64 (s, CH<sub>3</sub>), 24.16 (d, <sup>2</sup>J<sub>C,P</sub> = 3.5 Hz, PCH<sub>2</sub>CH<sub>2</sub>), 24.76 (d, <sup>1</sup>J<sub>C,P</sub> = 134.2 Hz, PCH<sub>2</sub>), 24.79 (s, CH<sub>3</sub>C=O), 75.56 (d, <sup>3</sup>J<sub>C,P</sub> = 16.7 Hz, CH), 82.57 (C<sub>q</sub>), 215.88 (CH<sub>3</sub>C=O) ppm.

**Exact mass** (ESI-MS): calculated for C<sub>7</sub>H<sub>15</sub>NaO<sub>6</sub>P<sub>1</sub> [M+Na]<sup>+</sup>: 249.0504, found: 249.0505.

## 4.2. BIOLOGICAL EVALUATION OF THE ANTIMALARIAL ACTIVITY

### 4.2.1. DXR INHIBITION ASSAY

The assay was performed in a reaction mixture containing 100 mM Tris HCl (pH 7.5), 0.2 % BSA, 1 mM MnCl<sub>2</sub>, 1 mM NADPH, 0.3 mM DOXP, and 1  $\mu$ g/ml recombinant DOXP reductoisomerase from *E. coli*. The mixture was incubated with a dilution series of the test compounds on a 96 well plate, and the reaction started by addition of DOXP. The decrease of absorption was monitored at 340 nm using a SpectraMax 340PC microplate reader (Molecular Devices, Ismaning).

#### **4.2.2. P. FALCIPARUM GROWTH INHIBITION ASSAY<sup>262</sup>**

##### **In vitro culture of *P. falciparum***

The *P. Falciparum* culture takes place in RPMI 1640-Medium, which is completed with 10% human serum and HEPES buffer.<sup>263</sup> Human Erythrocytes serve as hostcells. For the preservation culture Petri plates with 10 cm diameters are used. The culture volume per bowl amounts to 10 ml with a hematocrit of 5%. The cultures are preserved under an atmosphere of 5% O<sub>2</sub>, 3% CO<sub>2</sub> and 92% N<sub>2</sub> and by 37 °C. The medium is daily changed, and the cultures are diluted when reaching a parasitemia of maximally 5%. The parasitemia is determined microscopically with a Giemsa colored blood swab.

##### **Determination in vitro antimalaria activity**

Attempts for the determination of the in vitro antimalaria activity are accomplished on a 96 well plate.<sup>264,265</sup> Each well is filled with infected erythrocytes (200 µl per well, with 2% hematocrit and 0.4% parasitemia). Subsequently, serial dilution of the test substances are add to the wells. The substances are first solved in DMSO and dilute with complete culture medium. The plates are incubated first for 48 hours. After the addition of 0.8-µCi [<sup>3</sup>H]-hypoxanthine in 30 µl of medium per well, the plates were further incubated for 24 hours. The parasites are filtered on glass-fiber filters (Micromate 196, Packard). Incorporated radioactivity was counted with a β-Counter (matrix 9600, Packard). The only difference of this protocol to the Desjardins protocol is that we were incubating the parasites for 2 days instead of 1 day before adding labelled hypoxanthine. This made the assay more sensitive.

##### **Determination in vivo antimalaria activity**

The determination of the in vivo antimalaria activity takes place in a *P. vinckei* infected Balb/c mice model according to a modified protocol of Peters.<sup>266</sup> On day 0 the mice are infected through i.p. injection of approx.  $5 \times 10^7$  erythrocytes from the

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<sup>262</sup> Gorlitzer, K., Gabriel, B., Jomaa, H., Wiesner, J., *Pharmazie* **2006**, 61, 278-84. German.

<sup>263</sup> Trager, W., Jensen, J. B. *Science* **1976**, 193, 673-675.

<sup>264</sup> Ancelin, M. L., Calas, M., Bompard, J., Cordina, G., Martin, D., Ben Bari, M., Jei, T., Druilhe, P., Vial, H. J. *et al. Blood* **1998**, 91, 1426-1437.

<sup>265</sup> Desjardins, R. E., Canfield, C. J., Haynes, J. D., Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, 16, 710-718.

<sup>266</sup> Peters, W. *Malaria*, Kreier, J. P. Ed. (Academic Press, New York, 1980), vol. 1, pp. 160-161.

blood of an infected donor mouse. The treatment takes place on days 1, 2 and 3 through i.p. or p.o. application of the test substance in a volume of each time 0.2 ml. The substance is dissolved in a concentration of 150 mg/ml in DMSO and before the application diluted with H<sub>2</sub>O. For each group of doses 3 mice are used. On day 4 the parasitemia is counted out with a Giemsa colored blood swab.





*Chapter*



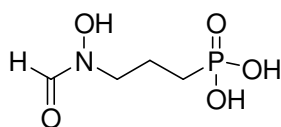
# CONCLUSIONS AND PERSPECTIVES



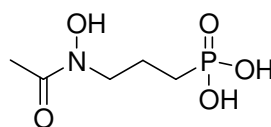


## 5. CONCLUSIONS AND PERSPECTIVES

This Ph.D. study constitutes part of an extensive project dealing with the design and development of new agents with antimalarial activity. The enzyme 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), which is involved in the DOXP pathway for isoprenoid synthesis, was chosen as drug target. This non-mevalonate pathway is not operating in humans and inhibitors of DXR can therefore be considered as selective for parasites. Fosmidomycin and its methyl homologue FR900098, well-known inhibitors of this enzyme that show antimalarial activity *in vivo*, were used as lead compounds for inhibitor design.



Fosmidomycin (1.83)



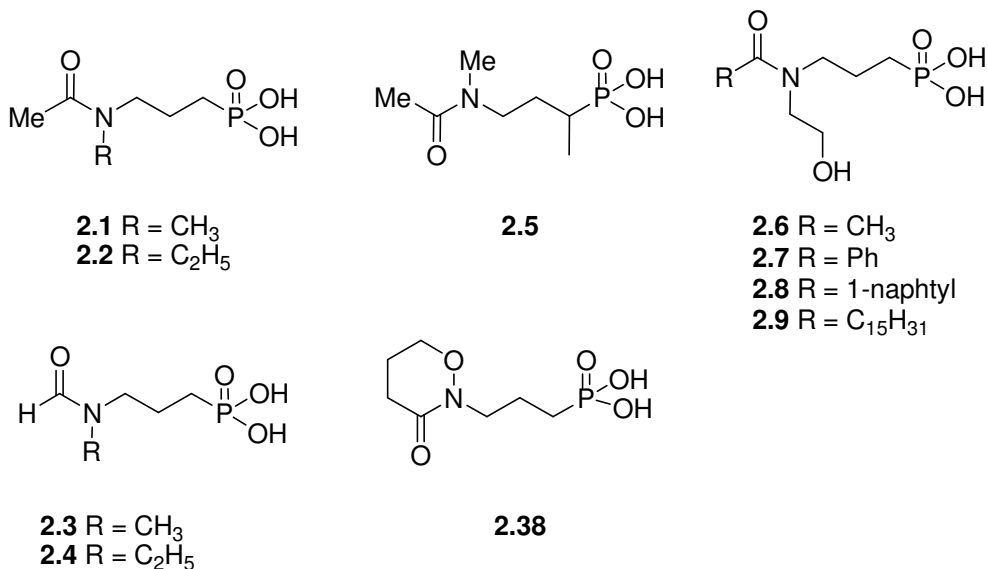
FR900098 (1.84)

The structure of fosmidomycin is characterized by a retrohydroxamic acid group, which acts as a ligand for the metal ion in DXR, and a phosphonate group, which mimics the phosphate group in the substrate structure. It was our objective to further expand the structure-activity relationship (SAR) of fosmidomycin as DXR inhibitor and to discover analogues showing superior activity against the enzyme and/or better antimalarial activity.

Modifications were applied to the retrohydroxamic acid group and the propyl chain of the parent structures. Synthetic pathways to these modified fosmidomycines were elaborated. As initial screening method all new compounds were tested for their ability to inhibit DXR of *Escherichia coli*. The most promising inhibitors were further tested in an *in vitro* assay on two different *Plasmodium falciparum* strains.

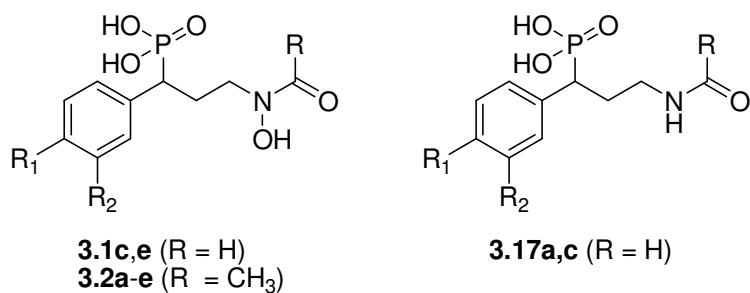
Our investigations are discussed in 4 chapters.

1. To explore the impact of structural modifications of the hydroxyl group of the retrohydroxamic moiety, analogues were synthesized with methyl, ethyl or hydroxyl ethyl substituents on the nitrogen (**2.1-2.9**).



These modifications did not produce any acceptable inhibitors, which demonstrates the importance of the hydroxyl group in ligating the divalent metal.

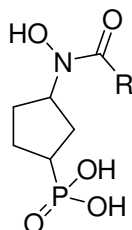
2. A synthetic procedure for the preparation of  $\alpha$ -aryl-substituted fosmidomycin analogues was developed starting from the corresponding benzylphosphonates or the appropriate cinnamaldehydes.



- a:** R<sub>1</sub> = H; R<sub>2</sub> = H  
**b:** R<sub>1</sub> = Me; R<sub>2</sub> = H  
**c:** R<sub>1</sub> = OMe; R<sub>2</sub> = H  
**d:** R<sub>1</sub> = Cl; R<sub>2</sub> = H  
**e:** R<sub>1</sub> = Cl; R<sub>2</sub> = Cl

Seven analogues were synthesized, allowing to perform an initial SAR study of this  $\alpha$ -aryl series (**3.1c,e** and **3.2a-e**). Although the  $\alpha$ -aryl analogues were generally weaker DXR inhibitors than fosmidomycin, these analogues unambiguously surpassed the activity of fosmidomycin in inhibiting *P. falciparum* growth. Remarkably, the formyl analogues **3.1c** and **3.1e** consistently outperformed the acetyl derivatives **3.2c** and **3.2e**, both in the enzyme and the parasite growth inhibition assays. Compound **3.1e** emerged as the most promising analogue with an  $IC_{50}$  value of 0.059  $\mu$ M. Due to their straightforward synthesis these compounds are suitable for upscaling in further drug development programmes. The deoxygenated sideproducts **3.17a,c** demonstrated the contribution of the hydroxyl group to the overall affinity.

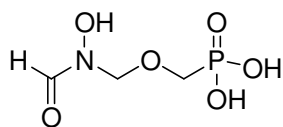
The Michael addition procedure, used to prepare the  $\alpha$ -substituted analogues from phenyl-substituted cinnamaldehydes, proved also valuable to prepare the fosmidomycin cyclopentyl analogues **3.3** and **3.4**.



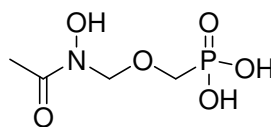
*trans*-**3.3** (R = H)  
*cis*-**3.3** (R = H)  
*trans*-**3.4** (R = CH<sub>3</sub>)  
*cis*-**3.4** (R = CH<sub>3</sub>)

These compounds, featuring a restricted mobility, showed that a *trans* orientation of the retrohydroxamic acid moiety and the phosphonate group is optimal for binding the enzyme.

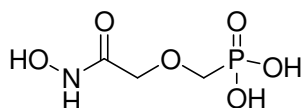
3. An efficient and reliable synthesis was established for  $\beta$ - and  $\gamma$ -oxa fosmidomycin analogues.



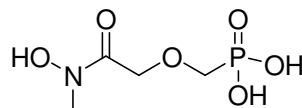
**4.1**



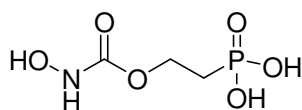
**4.2**



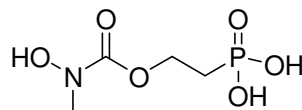
**4.3**



**4.4**



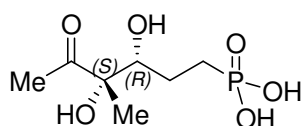
**4.5**



**4.6**

Analogue **4.4**, which combines a  $\beta$ -oxa modification with a hydroxamate moiety was almost as potent in inhibiting DXR as FR900098. In an *in vitro* assay it proved almost twice as active as FR900098 in inhibiting the growth of a *P.falciparum* 3D7 strain.

4. Several synthetic pathways towards the transition state analogue **5.1** were explored. A 10-step sequence, involving an asymmetric dihydroxylation, afforded compound **5.1** in a reasonable yield.



**5.1**

Unfortunately, this compound did not show any worth mentioning inhibitory activity.

In conclusion, this Ph.D. thesis highlights some important structural requirements for DXR inhibitors. This study also provides valuable information for modifications on fosmidomycin toward new and more efficient inhibitors: introducing aryl modifications on the  $\alpha$ -position of the phosphonate moiety significantly improves the antiplasmodial activity *in vitro* and one oxa analogue with a hydroxamic acid proved to be a more

potent inhibitor and to show superior antiplasmodial activity than the parent compound, fosmidomycin.

Further investigation on analogues **3.1e** and **4.4** is desirable since these compounds are significantly more potent than fosmidomycin on *P. falciparum* strains. Especially the latter one is very interesting, since it can be easily synthesized. Before further development the toxicological properties of these analogues need to be investigated and their pharmacokinetic profile needs to be established. *In vivo* studies, employing murine and monkeys models, are required. Further drug design may involve other sterically hindered analogues as well as prodrugs of the most promising analogues.



*Chapter*

# BESLUITEN EN PERSPECTIEVEN

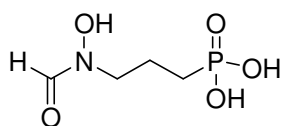




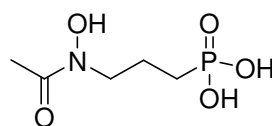


## 6. BESLUITEN EN PERSPECTIEVEN

Dit doctoraatsonderzoek maakt deel uit van een omvangrijk project omtrent het design en de ontwikkeling van nieuwe agentia met antimalaria activiteit. Het enzyme 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), dat betrokken is in de DOXP pathway voor isoprenoïde biosynthese, werd gekozen als target. Deze mevalonaat onafhankelijke “pathway” is niet actief in mensen en inhibitoren van DXR kunnen daardoor beschouwd worden als selectief tegen parasieten. Fosmidomycine en zijn methyl analoog FR900098, bekende inhibitoren voor dit enzyme die antimalaria activiteit vertonen *in vivo*, werden gebruikt als “lead” componenten voor het design van nieuwe inhibitoren.



Fosmidomycin (1.83)



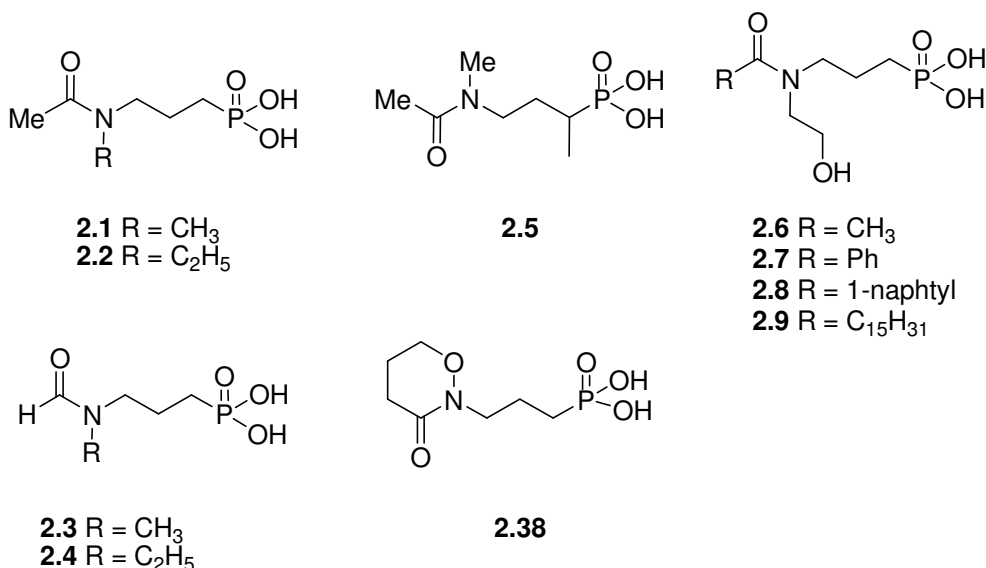
FR900098 (1.84)

De structuur van fosmidomycine wordt gekenmerkt door een retrohydroxamzuur, welke dienst doet als ligand voor het metaal ion in DXR, en een fosfonaat groep, welke de fosfaat groep in de substraat structuur nabootst. Het was onze doelstelling om de structuur-activiteitsrelatie (SAR) van fosmidomycine als DXR inhibitor verder uit te breiden en om nieuwe analogen te ontdekken die een hogere activiteit tegen het enzyme en/of een betere antimalaria activiteit vertonen.

Modificaties werden aangebracht aan de retrohydroxamzure groep en de propylketen van de twee lead componenten. Verschillende synthese wegen werden uitgewerkt voor deze gemodificeerde fosmidomycine analogen. Als initiële screeningsmethode werden alle nieuwe componenten getest op hun vermogen om DXR van *Escherichia coli* te inhiberen. De meest beloftevolle inhibitoren werden vervolgens getest in een *in vitro* test op twee verschillende *Plasmodium falciparum* stammen.

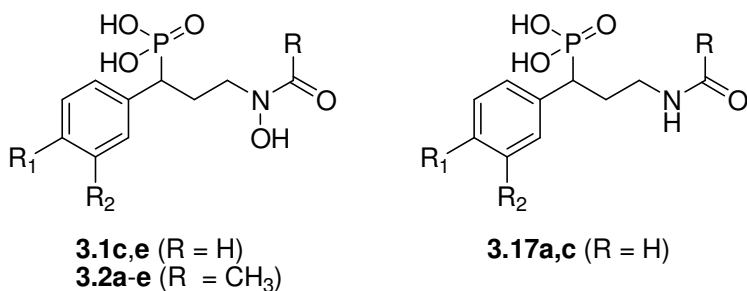
Ons onderzoek wordt in vier hoofdstukken besproken.

1. Om de impact van structurele veranderingen van de hydroxyl groep van de retrohydroxamzure groep na te gaan, werden analogen gemaakt met een methyl-, ethyl- of hydroxyethyl substituent op de stikstof (**2.1-2.9**).



Deze modificaties resulteerden niet in aanvaardbare inhibitoren, hetgeen het belang van de hydroxyl functie aantoont in de binding van het divalent metaalion.

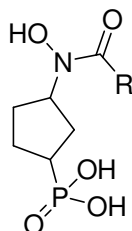
2. Een synthetische procedure voor de aanmaak van α-aryl gesubstitueerde fosmidomycine analogen werd ontwikkeld. Deze procedure startte van de betreffende benzylfosfonaten of de geschikte cinnamaldehydes.



- a:** R<sub>1</sub> = H; R<sub>2</sub> = H  
**b:** R<sub>1</sub> = Me; R<sub>2</sub> = H  
**c:** R<sub>1</sub> = OMe; R<sub>2</sub> = H  
**d:** R<sub>1</sub> = Cl; R<sub>2</sub> = H  
**e:** R<sub>1</sub> = Cl; R<sub>2</sub> = Cl

Zeven analogen werden gemaakt, hetgeen ons toeliet een initiële SAR studie van deze  $\alpha$ -aryl analogen (**3.1c,e** en **3.2a-e**) uit te voeren. Alhoewel deze analogen allemaal zwakkere DXR inhibitoren dan fosmidomycine waren, vertoonden ze toch een ontegensprekelijke betere activiteit in de inhibitie van *P. falciparum* groei dan fosmidomycine. Opmerkelijk is dat de formyl analogen **3.1c** en **3.1e** duidelijk de acetyl derivaten **3.2c** en **3.2e** overtroffen, zowel in de enzyme als de parasietgroei inhibitietest. Component **3.1e** kwam naar voor als het meest beloftevolle analoog met een  $IC_{50}$  waarde van 0.059  $\mu$ M. De ontwikkelde synthesesmethode is geschikt voor opschaling naar verdere geneesmiddelontwikkeling toe. De minder actieve gedesoxygeneerde nevenproducten **3.17a,c** tonen de bijdrage van de hydroxyl groep voor de totale activiteit aan.

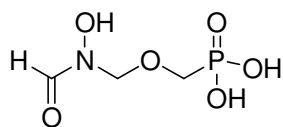
The Michael additie die gebruikt werd om de  $\alpha$ -gesubstitueerde analogen te maken vanuit de phenyl gesubstitueerde cinnamaldehydes, bleek ook een beproefde methode om cyclopentyl fosmidomycine analogen **3.3** en **3.4** te maken.



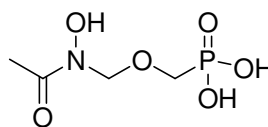
*trans*-**3.3** (R = H)  
*cis*-**3.3** (R = H)  
*trans*-**3.4** (R = CH<sub>3</sub>)  
*cis*-**3.4** (R = CH<sub>3</sub>)

Deze componenten met een gereduceerde flexibiliteit van de propylketen, maakten duidelijk dat een *trans* orientatie van het retrohydroxamzuur en de fosfonaat groep optimaal is voor de binding aan het enzyme.

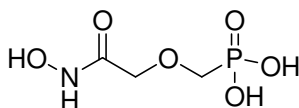
3. Een efficiënte en betrouwbare synthese werd ontwikkeld voor  $\beta$ - en  $\gamma$ -oxa fosmidomycine analogen.



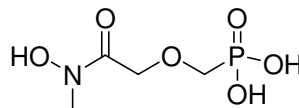
4.1



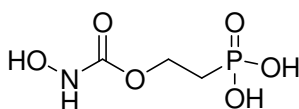
4.2



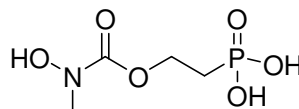
4.3



4.4



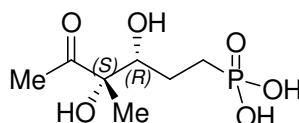
4.5



4.6

Analoog **4.4**, welke een  $\beta$ -oxa modificatie combineerd met een hydroxamaat groep, bleek bijna even potent in het inhiberen van DXR als FR900098. In een *in vitro* test bleek het bijna twee maal zo actief als FR900098 te zijn in het inhiberen van de groei van een *P. falciparum* 3D7 stam.

4. Verschillende pogingen tot de synthese van een transitietoestand analoog **5.1** werden ondernomen. Een 10-staps sequentie, met een asymmetrische dihydroxylatie, leverde component **5.1** in een aanvaardbaar rendement.



5.1

Helaas vertoonde dit analoog een niet noemenswaardige inhibitie.

Als besluit kunnen we stellen dat dit doctoraatswerk enkele belangrijke structurele eigenschappen voor DXR inhibitoren aantoont. Deze studie levert ook waardevolle informatie voor modificaties aan fosmidomycine met het oog op nieuwe en meer efficiënte inhibitoren. Zo verbetert de introductie van aryl modificaties op de  $\alpha$ -positie

van de fosfonaat groep opmerkelijk de antiplasmodiale activiteit *in vitro*. Ook bleek een oxa analoog met een hydroxamzuur een meer potente inhibitor en toonde een sterkere antiplasmodiale activiteit dan de moeder molecule, fosmidomycine.

Verder onderzoek op analogen **3.1e** and **4.4** is wenselijk, aangezien deze componenten significant meer potent zijn dan fosmidomycine op *P. falciparum* stammen. Vooral de laatste is zeer interessant omdat het gemakkelijk gesynthetiseerd kan worden. Alvorens verdere ontwikkelingen te plannen moeten de toxicologische eigenschappen van deze analogen onderzocht worden en moet hun farmacokinetisch profiel bekeken worden. *In vivo* studies die gebruik maken van knaagdier- en aapmodellen zijn noodzakelijk. Verder geneesmiddelen design kan o.a. zowel sterisch gehinderde analogen als “prodrugs” van de meest beloftevolle analogen inhouden.



**2.1** R = CH<sub>3</sub>  
**2.2** R = C<sub>2</sub>H<sub>5</sub>

**2.3** R = CH<sub>3</sub>  
**2.4** R = C<sub>2</sub>H<sub>5</sub>

**2.5**

**2.6** R = CH<sub>3</sub>  
**2.7** R = Ph  
**2.8** R = 1-naphthyl  
**2.9** R = C<sub>15</sub>H<sub>31</sub>

